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# ORIGINAL ARTICLES

EFFECTS OF EARLY EARTHING-UP OF SUGARCANE ON THE ATTACK OF THE STEM BORER, ARGYRIA STICTICRASPIS HMPSN.

By B. D. Gupta, M.Sc., Assoc. I.A.R.I., Sugarcane Research Station, Muzaffarnagar (Received for publication on 25 August 1943)

EARTHING-UP of sugarcane plants during rains has been recommended by Sethi, et al. [1937] as a normal cultivation procedure to prevent the crop from lodging both when planted in trenches or on flat.

Early earthing-up as a measure to control the damage done to shoots by the sugarcane moth borer, Argyria sticticraspis Hmpsn, was recommended for the first time by Subramaniam and Ramiah The experiments conducted at Government Sugarcane Farm, Mandya (Mysore State), showed that by a light earthing-up, the soil on both sides of the cane rows is thrown over the young shoots and when this is followed by irrigation, the soil settles down in the space between the first leaf-sheath and the plant stalk. This operation prevents the borer from living as a leaf miner in the first leaf sheath prior to its entry into the cane stalk. The method of control was observed at Mandya to reduce borer attack by more than 50 per cent without in any way interfering with the tillering and vigour of the crop.

On the basis of this recommendation, experiments were taken up at the Sugarcane Research Station, Muzaffarnagar, to study the effects of early earthing-up on the incidence of A. sticticraspis, the most serious of the sugarcane borers during the hot weather months of May and June. Studies were also carried out on the effects of early earthing on tillering, cane formation, yield and maturity of the crop. The results obtained during three crop seasons, 1937-38, 1939-40 and 1940-41 are given in the present paper.

#### EXPERIMENTAL

The planting of sugarcane in the western districts of the United Provinces is generally done during March. The shoots of the new crop appear during the fourth week, after planting. It is after the middle of April that A. sticticraspis migrates to plant crop, after completing its first brood in the ratoon crop during March and April. The drying up of the central leaf-sheath is the first sign of the commencement of borer attack. The borer infestation up to the middle of May is confined to a small percentage of plants, varying from 2 to 8 per cent. After this period the infestation increases with the progress of the hot weather and continues to be heavy upto the middle of July, when it begins to decline and gradually

dies off with the progress of the monsoon rains in

August.

Preliminary observations on the various effects of early earthing-up were made in 1936 on a nonreplicated plot basis. One earthing by the middle of May brought down borer attack by 12 per cent in comparison to crop earthed-up in August. The tillering was reduced by 50 per cent canes formed were less by 28 per cent and vield by 10 per cent in comparison to the normal crop. The individual weight of sticks in the earthed-up plot increased by about 20 per cent and there was no material effect on the sugar contents. It was also observed that the irrigation after earthing-up washed down most of the soil raised up to protect the tender stalks of the plants from borer attack. In subsequent field trials, laid out in randomized blocks with four replications each year, earthing was carried out at the time of hoeing after irriga. tion. Three, two and one earthing followed from the middle of May, middle of June and middle of July, respectively, were compared with no earthing during 1937-38 and 1939-40. During 1940-41 the interval between various earthings was reduced from one month to a fortnight in the treated plots, with the result that the various earthings finished by the middle of June and in order to prevent the crop from lodging during rains, a final earthing-up was carried out in August in all the plots.

The incidence of borer attack was recorded by making counts of all the healthy and infested shoots in the four replications of the treated and control plots. The size of an individual plot was 1/27.4 acre in 1937-38 and 1/23.3 acre in 1939-40 and 1940-41. The observations were recorded before the first earthing was carried out and were repeated at fortnightly intervals till the middle of September when the borer attack almost disappeared. The 'dead hearts' or the central whorl of dried-up leaves were pulled out from the infested plants, in such a way, that the borer was left behind in the plant stalk. The method of digging out the infested plants was not adopted as it would have interfered with the natural inci-

dence.

EFFECT OF EARTHING ON BORER INCIDENCE
The effect of earthings on the borer incidence
is shown in Table I. The records of borer infestation include a short percentage of the root borer

[XV

Table
Early earthing and

			<del>,</del>			eartning and
					Nu	mber of shoots
Crop <b>se</b> ason	Treatment	Dates of earthings	Variety	Up to 15 May	From 16 May to- 15 June (1st round)	Decrease in borer attack compared to unearthed-up plots
1	2	3	4	5	6	7
1937-38.	A-Three earthings	16/5, 16/6 and 16/7	Co. S. 70	356	9124	44.1%
	B-Two " .	16/6 and 16/7 .	,,	329	15317	Unearthed-up
[발생시다]	C—One " .	16/7	p <b>y</b>	438	15289	,,,
남한왕왕왕 왕고 12일 12일 왕왕왕 12일 12일	D—Control .	••		301	18385	<b>"</b>
Critical difference at 5 per cent level						.,.
1939-40	A—Three earthings	16/5, 16/6 and 16/7	Co. S. 70	2004	8062	34 · 6%
	B-Two " .	16/6 and 16/7 .	10	1072	11324	Unearthed-up
	C-One ".	16/7	<b>"</b>	1328	12302	<b>,</b>
	D—Control .			1351	13347	
Critical difference at 5 per cent level						•••
1940-41	A—Three earthings	16/5, 1/6 and 16/6	Co. S. 178	1538	9005	<b>4</b> 1·01%
	B—Two ,, .	1/6 and 16/6	,	1491	9926	35.01%
	C-One ".	16/6	,	1678	15448	Unearthed up
	DControl .			2027	15098	
Cutton Pales						
Critical difference at 5 per cent level		•••				

borer attack

From 16 June to 15 July (2nd round)  8	Decrease in borer attack compared to unearthed up plots	From 16 July to 15 August (3rd round)	Decrease in borer attack compared to unearthed up plots	After 15 August	Total No. of shoots destr. yed per acre from May to September	Per cent decreas in borer attack due to earthing-up
4904		10	11			
	19 · 2%			12	13	14
	19.2%	1				The state of the s
4070		1151	4 6%		15536	40.7%
4658	23.3%	1096	9.1%		21399	18.3%
5836	Unearthed-up	1370	-13.6%		22933	12.4%
6302		1206	Unearthed-up		26194	•••
				•••	5453	
5592	14·4%	1165	10.7%	140	16962	18.8%
5452	16.6%	1235	5.4%	92	19176	8.1%
6338	Unearthed-up	1561	-19.2%	92	21622	—3·5%
6734	,,	1305	Un-earthed-up	140	20877	
		•••		•	Insignificant	
4893	44.4%	746	23 · 6%	163	16333	39.9%
3961	55.02%	489	50.0%	140	16007	41.1%
5219	40.7%	489	50.0%	140	22981	15.4%
8807	Unearthed-up	978	Un-earthed-up	256	27168	
					1636	

Table Effect of early earthing

					Ti	llers
Crop season	Variety	Treatments	Date of planting	Per cent germination middle of May	Per acre on 15 July	Per cent increase or decrease in comparison to control
1	2	3	4	5	6	7
1937-38	Co. S. 70	A—Three earthings	22/3/1937	40 4	87433	12.9%
		B—Two ,, . C—One ,, .		39·1 41·2	91653 96558	8.7%
The state of the s		D—Control		40.8	104284	
Critical difference at 5 per cent level	•	••••	•••		8334	
1939-40	Co. S. 70	A—Three earthings	10/3/1939	40·4 36·4	73558	12.3%
	),	B—Two ,, . C—One ,, . D—Control .		39·9 35·0	77007 87165 80548	8.2%
Critical difference at 5 per cent level					6149	•••
1940-41	Co. S. 178	A—Three earthings B—Two ". C—One ".	16/2/1940	41·0 40·5 44·9	52564 48370	25·5% 31·4%
	<b>,</b>	D—Control		45.8	66428 70552	5.8%
Critical difference at 5 per cent level					2584	

II on the crop

	Canes	Y	ield per acre	J	Juice analysis
Formed per acre	Per cent increase or decrease in comparison to control	In maunds	Per cent increase or decrease in compari- son to control	Per cent sucrose in juice	Purity coefficient
8	9	10	11	12	13
					And the state of t
57303	15/10/37 8 · 6	657 · 1	20/4/38 8 5	12.4	20/4/38 76.00
60280	5.6	707.5	16.9	13.9	80.2
62145	2.7	666.4	10.1	12.5	76.4
63842		605.3		10.5	70.3
					Marine Marine Marine Parrick Miller, April 20 September 1995 Consultation of the Consu
3444		Insignificant	•••		
41450	31/1/40 4 · 2	799 · 8	31/1/40 13.3	13.0	1/2/40 76 · 4
42140	5.9	789 · 3	11.8	12.9	75.7
41965	5.5	753 · 4	6.7	13.0	76 · 3
39795		706.0		13 · 2	77.1
nsignificant		60.9			지 (1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1
31455	8/2/41 11:2	453.0	9/9/41 1.0	16.5	16/9/41 96.9
30499	13 9	458·0	8/2/41 1·9 0·9	16.5	16/2/41 86·3 87·4
33552	5.3	482.8	4.5	16 · 4	87.3
35439		462.0		17:1	87-9
1682	<b></b>	Insignificant	•		

Emmalocera depressella Swinh, which in the early part of the season occur much in the same way as the larvae of A. sticticraspis and cannot be easily differentiated from each other from the nature of

the 'dead hearts'.

A reference to Table I will show that 20 to 27 thousand shoots per acre are destroyed by the dead heart borers, during the crop season, from May to September. The greatest damage to the shoots occurs during the period of about a month, from the middle of May to the middle of June. One earthing carried out by 16 May went a long way to protect the plants from borer attack. It was observed on 15 June, prior to the second earthing-up that there was a decrease in the number of damaged shoots in the earthed-up crop (Treatment A) by 44 per cent in 1937 and 35 per cent in 1939 when compared with the unearthedup crop in treatments B, C and D. During this period two earthings were given, first on 16 May and second on I June in treatment 'A' and one earthing on I June in treatment 'B' of 1940 crop. This resulted in a decrease in borer attack by 41 and 35 per cent respectively in comparison to unearthed-up crop in treatment 'C' and 'D'.

In the second round, i.e. between the middle of June and middle of July, the intensity of attack was 50 per cent less as compared to the first round. An earthing carried out by the middle of June in plots earthed-up in May (Treatment 'A') showed on 15 July a decrease in the number of borerdamaged shoots by 19·2 per cent in 1937 and by 14·4 per cent in 1939, in comparison to treatments 'C' and 'D' which were unearthed-up. A decrease by 23.3 per cent in 1937 and 16.6 per cent in 1939 was recorded in treatment 'B' where earthing was carried out for the first time on 16 June. By this time, three, two and one earthings had been given in the 1940 crop where a decrease in the number of damaged shoots by 44.4,  $55 \cdot 2$  and  $40 \cdot 7$  per cent was recorded in treatments A, B and C respectively, in comparison to the corresponding damage in the unearthed-up

The borer attack during the period from the middle of July to the middle of August or afterwards is not severe as shown in Table I, treatment 'D' column 10. An earthing carried out on 16 July in twice earthed-up plots (Treatment A) showed a decrease in borer attack by 4.6 per cent in 1937 and 10.7 per cent in 1939, while in plots earthed-up but once (Treatment B) there was decrease by 9.1 per cent in 1937 and 5.4 per cent in 1939. An earthing carried out for the first time on 15 July in Treatment 'C' in 1937 and in 1939 did not bring about any material decrease in borer attack. A decrease varying from 23.6 to 50 per cent was recorded on 15 July in earthed-

up plots (Treatments A, B and C) in 1940 in comparison to control.

EFFECT OF EARLY EARTHING-UP ON THE CROP

(i) Tiller formation. Earthings were commenced by the middle of May when about 40 per cent of the eye buds planted had germinated (Table II, column 5). As a result of two earthings, one on 16 May followed by another on 16 June in Treatment A (Table II, column 7), tillering decreased by about 13 per cent in July in comparison to crops in treatments C and D where no earthing was carried out till the middle of July in 1937 and in 1939. In these two years reduction in the number of tillers was about 8 per cent in treatment B where only one earthing was resorted to on 16 June. During 1940 three earthings were completed by the middle of June in treatment A, two in treatment B and one in treatment C, at fortnightly intervals, as a result of which tillering was reduced by 25 to 31 per cent under three and two earthings and by about 6 per cent under one earthing when compared to control or unearthed-up crop. This is contrary to the findings of Subramaniam and Ramiah [1935] at Mandya where light earthing had no adverse effect on tillering, but agrees with the findings in Bihar as stated by Sethi [1940].

(ii) Cane formation. The significant interference in the tillering capacity is not limited to the young shoots alone but brings about a reduction in the number of canes formed in comparison to the crop where normal earthing is carried out during rains or where the crop is left unearthed.

During 1937-38 a decrease by 3, 6 and 9 per cent was observed in the number of canes formed per acre, respectively, under once, twice and thrice earthed-up crop in comparison to the crop which was not earthed-up. This reduction in the number of canes formed per acre varied from 5 to 14 per cent in 1940-41 and confirmed the observations made during 1937-38. It was in 1939-40 that the number of canes formed in the treated area increased by about 5 per cent compared to the unearthed-up crop. This increase in the number of canes was found to be insignificant on statistical analysis. Besides, it was observed that the average weight of the individual cane sticks, from the earthed-up crop, had slightly increased, and was proportionate with the number of earth-

(iii) Yield. An increase in yield from 50 to 100 md. per acre was observed in the earthed-up crop of Co S 70 during 1937-38 and 1939-40. But in 1940-41 when the experiment was repeated with Co S 178, one to two per cent decrease in the yield of sugarcane occurred under three and two earthings, though one earthing resulted in

about  $4\frac{1}{2}$  per cent increase over the unearthed-up crop.

(iv) Juice quality. It has been observed by Sethi, et al. [1937] that the percentage of sucrose and purity coefficient in juice slightly decreases on earthing up. This is confirmed from juice analvsis carried out at the time of harvest by taking two clumps from each plot and analysing the composite samples from each treatment. It would be observed from Table II, columns 12 and 13, that there is a slight decrease in the per cent sucrose and purity coefficient in the juice analysis data of Co S 70 for 1939-40. It is confirmed in the subsequent year from the juice analysis results of Co S 178, where the decrease in the sucrose percentage is from half to one per cent. The year 1937-38 happened to be rather an abnormal year on account of a very severe attack of pyrilla, as a result of which the crop remained considerably poor in sugar contents, even as late as in April. The unearthed-up crop was poorer still as it had badly lodged.

#### SUMMARY AND CONCLUSIONS

Light earthing-up, when the crop is young, has been found to be a useful measure in Mysore State as it protects the tender stalks of plants from the ravages of stem borer A. sticticraspis. This method of checking the borer damage to sugarcane plants was given a fair trial at Sugar-

cane Research Station, Muzaffarnagar, during 1937-38, 1939-40 and 1940-41.

Three, two and one earthing commenced from the middle of May and repeated at monthly and fortnightly intervals were compared with the unearthed-up crop. The various effects of early earthing-up on stem borer attack, tillering, cane formation, quality and quantity of the crop, were studied. It was observed that two to three earthings during May and June considerably reduced the borer attack. Tillering and cane formation is lowered but without materially interfering with the yield and quality of cane at harvest. Two light earthings, one by the end of May and another by the middle of June followed by the final earthing-up during rains, are recommended.

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## THE ANALYSIS, GRADING AND UTILIZATION OF INDIAN LINTERS

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#### I. G 11::.

#### Introduction

COTTON linters are those short fibres which are left on the seed after the lint has been removed in the ginning operation. These are subsequently extracted by a special process called delinting. These linters are now-a-days used in numerous industries such as the manufacture of artificial silk, insulators, radio panels, non-shatter glass, bottle caps, cellulose acetate and nitrate lacquers, explosives, etc., and thus represent a valuable raw material in the economy of a country. Though linters appeared as an industrial raw material in United States of America about the end of the nineteenth century, production of linters on a commercial scale was unknown in India until very recently when the Indian Central Cotton Committee began to take

an interest in the problem. At present a few thousand bales of linters are produced annually in this country, but in the absence of any cellulose industry in India, the producer has to depend on the foreign markets in which he meets with strong competition from the highly organized American linters industry. Consequently only a portion of the total material potentially available is at present recovered, while the remainder is disposed of with the seed and thus represents a clear loss to the country.

Owing to the non-uniform quality of Indian linters, as will be seen from data presented in this report, considerable difficulty has been experienced in disposing of even the present small supply of linters. Therefore, if Indian linters are to be put to their numerous industrial uses within or outside the country, it is necessary that they should be standardized in different grades. Grading would

not only help in finding a reliable market for the raw material, but would pave the way for building up a new industry, namely the chemical cotton industry in this country, which is the starting point

for numerous other industries.

Any sample of linters contains a certain quantity of trash associated with it, which is made up of dust, leaf rests, stalk-bits, boll husks, cut and uncut seed, etc. The larger the quantity of this trash or foreign matter, the lower in general is the grade of the sample, though the type or nature of the trash would also influence the grade to some extent. The estimation of the pure cellulose in a commercial sample can be carried out with a high degree of accuracy by means of a kier boiling and bleaching treatment and subsequent analysis, while the amount of trash can be approximately found by passing the sample through a suitable opening and cleaning machinery. The real basic raw material of the cellulose industry, which consumes about two-thirds of the available supply of linters, however, is alpha-cellulose which is prepared from the former by chemical methods. It was, therefore, decided that the alpha-cellulose content of the Indian linters should be ascertained experimentally and that these values should be correlated to the percentage of fibre and percentage of trash as found by mechanical cleaning, as the latter method can be used more conveniently for grading the linters.

In America the method of surface grading is followed for grading linters. Sets of seven standard grades of American linters are prepared by the Department of Agriculture, which are supplied to the purchasers on payment of a fixed sum. One such set of standard grades of American linters was obtained for the Technological Laboratory with a view to comparing Indian linters with the American linters. The results obtained by this method are also discussed in this report and compared with those obtained by the mechanical

analysis.

Material. The material to be investigated, viz. linters, belonged to the two seasons, 1938-39 and 1939-40 and was obtained either from the ginning factories or from oil mills, mainly situated in northern India. An attempt was also made to obtain information as regards the variety of seedcotton, the type of gin in which it was ginned and the type of delinting machine used in recovering the linters so as to correlate, if possible, these various factors. Information was also sought on the cost of production, the railway freight and the price of linters so as to take into account these economic factors, especially in the matter of comparison with the American linters. So far as 1938-39 samples are concerned the information on these various points was far from being complete, but it was more definite as regards 1939-40 samples.

Experimental procedure (A) Analytical and (B)
Surface grading

(A) The analytical methods used on linters were (1) mechanical cleaning and (2) chemical treatment.

(1) Mechanical cleaning. A sample of linters weighing 50 gm. was passed through the Shirley Analyser and the treatment was repeated on the trash until it ceased to deliver any appreciable quantity of fibre. The clean fibres thus obtained were found to be almost free from any impurities, but their quantity varied considerably according to the amount of trash present in the original sample. It should be stated that during the passage of the sample through the Shirley Analyser some of the very short fibres were lost in the blast and their loss has been designated as the 'invisible loss'. Furthermore, as it was not possible to ascertain the amount of dust in linters by this mechanical treatment, the ash content of the raw linters has also been determined as an index of dust content and has been tabulated along with the results of mechanical analysis.

(2) Chemical treatment. A sample of linters weighing about 50 gm. was given, without any mechanical cleaning except the removal of whole seed, an alkali treatment in an autoclave without a stirrer under conditions stated below:

10 per cent caustic soda on the weight of linters—1:10 bath ratio.

5 hr. duration of treatment—160° C. temperature.

Prior to alkali treatment the linters were wetted with 1 per cent solution of Lisapol A and the quantity of water retained by them was taken into account while fixing the bath ratio at 1:10.

Linters removed from the autoclave were washed clean with water and soured with 1 per cent acetic acid solution for  $\frac{1}{2}$  hr., before they were subjected to the bleaching treatment. The conditions of this treatment were kept uniform throughout this investigation. The soured sample was kept for two hours in a calcium hypochlorite solution with about 2 g/l available chlorine. The bath ratio of the linters to bleaching liquor was kept 1:20. On completion of bleaching the linters were washed with water, soured for 15 min. in a  $\frac{1}{2}$  per cent solution of hydrochloric acid, washed free of the acid and finally dried.

The chemical cotton thus obtained was tested for its alpha-cellulose content according to method described by Doree. The ash content of chemical cotton was also determined as it forms an important criterion of the quality of the material in

many industries.

(B) Surface grading. The American standards for the year 1939-40 were accepted as the basis for this study. A representative surface was prepared from each sample for comparison with standard American grades and the sample of Indian linters was allotted that particular number of the American grade with which it matched best in general character. In most cases the Indian linters did not match exactly with the American ones in every detail, and consequently it was found necessary to note down the salient points of difference.

#### II. RESULTS AND DISCUSSION

The results of the mechanical analysis of Indian linters of two seasons are given in Tables I (a) and I (b).

(a) Discussion of the results of mechanical analysis

In Tables I (a) and I (b) the results of mechanical analysis have been given in terms of fibre, trash, invisible loss and for the sake of convenience of comparison the values of ash percentages are also shown. These will now be dealt with individually.

Fibres. The wide variation in the fibre contents of the 1938-39 samples, ranging from 37.5 to 80 per cent show clearly the urgent necessity of standardizing Indian linters. Although the linters of 1939-40 season show a considerable improvement over those of the previous season in this respect, it is certain that further improvement would result from standardizing the Indian The fibre percentage does not represent the total quantity of fibres present in a given sample, as some of the very short ones may be blown off with the air-current, while others may remain in the trash in the form of neps and motes which are not opened up by the Shirley Analyser. For the present, however, the percentage of fibres as given in Tables I (a) and I (b) is accepted as a useful figure representing the quantity of fibres in each case.

Trash. A glance at the percentage of trash given by the mechanical cleaning will show that our delinting needs considerable improvement. In a number of cases, particularly in the 1938-39 season, the percentage of trash must be regarded as very high, denoting that either through lack of knowledge or care, the linters became adulterated with dust, seed and leaf rests. In fact, it is difficult to understand how leaf rests could find their way to-linters to such an extent. Furthermore, both cut and whole seed has been found in several samples of both the seasons which can only be due to carelessness or faulty delinting. Except in this respect, i.e. the presence of cut or whole seed, the linters of 1939-40 season show very considerable improvement over those of the previous season.

The green undeveloped seed which was often found in 1939-40 samples, must also be avoided. It will be shown later that it depresses the quality of chemical cotton by lowering the alpha-cellulose content. The necessity of properly cleaning the seed prior to delinting cannot be too strongly emphasized.

Invisible loss. This is caused mostly by some of the very short fibres being blown off with the air current and to a very small extent by the fine particles of dust escaping along with these short fibres. There is bound to be some fluctuation in the percentage of invisible loss as it would depend upon the quality of linters. It may be noted that the invisible loss is very considerable in case of two samples Nos. 1 and 9 in 1938-39 season and in one sample No. 8 in the following season. These apparently contained more short fibres or fuzz than the others.

The invisible loss was comparatively small for the 1939-40 samples showing thereby that the extremely short fibres or fuzz were not removed from the seed during delinting. It is a controvertible point whether this practice should be encouraged, as it obviously results in a lower yield of linters. In such cases it would perhaps be better to have recourse to two cuts instead of only one and market the produce separately. In any case this problem offers scope especially with regard to the economic factors.

Ash. The determination of ash content of raw linters was undertaken as an index of the amount of dust in them, as the separation of dust from the trash was not possible.

The ash content of raw linters of 1938-39 season fluctuates between 2 and 11·3 per cent. This is a wide range and shows that linters were not carefully handled. In fact, in two cases, viz. Nos. 8 and 12 the ash content is so high that it is difficult to explain it on the grounds of the natural impurities present in linters. Table I (a) shows that the high ash content cannot always be attributed to the presence of leaf or seed rests. There are samples which have a very high percentage of trash consisting mainly either of seed rests or leaf rests but give a low ash content, and vice versa. Hence, when an abnormally high ash content is found in raw linters, it is generally due to dust and sand particles.

Turning to the 1939-40 samples we find that except in two cases, namely Nos. 9 and 23, the ash content is always below 3 per cent which may be regarded as the maximum permissible limit, as generally speaking our experiments have shown that linters possessing less than 3 per cent ash content give a chemical cotton whose ash content is below 0.12 per cent which is the limit accepted for industrial chemical cotton. The need for

Table I (a)
Results of mechanical analysis of Indian linters, 1938-39 season

Serial No.	Seed	Station	Saw or Roller gin	Fibres per cent	Trash per cent	Invisible loss per cent	Ash per cent
				1			
	Sind-Hyderabad .	Hyderabad, Sind.	•	62.5	15.0	22.5	2.9
1	4F (Punjab)	Punjab		75.0	15.0	10.0	2.0
2	arr (Yunjab)	Sind		72.5	17.5	10.0	2.4
3	285F (N. T.) Sind .	Mirpurkhas (1)		65.0	22.5	12.5	3.7
4	285F (Mirpurkhas) .	,, (2) .		52.5	37.5	10.0	2.9
5	2007 (D 11)		••	80.0	12.5	7.5	3.0
6	289F (Punjab)	Punjab	Saw gin	75.0	15.0	10.0	5 · 2
7	" saw gin	Sargodha	Roller gin	57.5	32.5	10.0	9.7
8	", roller gin	,,	Saw gin	37.5	47.5	15.0	5.1
9	Mixed roller gin .		Saw gui	75.0	15.0	10.0	2.4
10	4F-75 per cent	Okara	•••	100	10 0		
	Desi-25 per cent \			67.5	20.0	12.5	4.1
11	4F-70 per cent	,,	• • • • • • • • • • • • • • • • • • • •	07-3	20 0	17 7	
	Desi-50 per cent						
12	289F—91 per cent			45.0	45.0	10.0	11.3
	4F-7 per cent	••	Roller gin	45.0	45.0	10.0	11 0
	L.S.S.—2 per cent			00.0	07 5	12.5	3.4
13	289F (saw gin) .		Saw gin	60.0	27.5	12.3	3 =
14	4F-76 per cent				00 7	10.0	4.4
	Desi-18 per cent }		• •	57.5	32.5	10.0	4.4
	289F-6 per cent					1. 1131 341	
15	289F—91 per cent	I di ili a ismilano					1 , -
-0	4F-7 per cent		Saw gin	70.0	20.0	10.0	4.5
	L. S. S.—2 per cent			The street of	A CONTRACTOR		

Table I (b)

Results of mechanical analysis of Indian linters 1939-40 season

Serial No.	Seed	Station	Saw or Roller gin	Fibres per cent	Trash per cent	Invisible loss per cent	Ash per cent
			vii.				
1	4F	Khanewal	Saw	81.0	11.6	7.4	$2 \cdot 1$
2		Mailsi	Saw	81 · 2	12.6	6.2	$1 \cdot 7$
		Vihari	Saw	78 · 2	14.2	7.6	2-4
3A	289F		Saw	83.0	9.8	7.2	1.6
3B		Okara	Saw	86.8	8.2	5.0	1.4
4	Desi-20 per cent	Okara	Daw	30 0	)		
	PA.—80 per cent \( \)		Saw	86 · 4	6.4	$7 \cdot 2$	1.4
5	Desi-50 per cent		Daw	00 <del>1</del>	0 1	• •	- 1 - 1 - 7 - 7
	PA.—50 per cent \( \)	TT 1 1 1 0'-1	G D-11	83 · 8	11.6	4.6	2.3
6	285F	Hyderabad, Sind.	Saw and Roller	93.9	11.0	<b>T</b> 0	- 0
	Bakaranah ka secia.		mixed	00.4	11.6	6.0	2 · 2
7	•	• •	<b>&gt;</b> >	82 · 4			$\frac{2}{2} \cdot \frac{2}{0}$
8		Navsari		70.6	14.0	15.4	3.8
9	4F	Burewala	Saw	75.0	18.0	7.0	
10	289F	Sargodha	Saw	82.0	9.4	8.6	$2 \cdot 1$
11	Desi-30 per cent	Okara	Saw	83 0	10.6	6.4	1.5
	PA.—70 per cent		[사용하다 이 교육에 1900 ] [				
12	289F	Mianchannu .	Saw	82.0	10.6	7.4	2 · 1
13	4F	Arafwala	Saw	76.4	18.0	5.6	2.5
14	4F	Montogmery .	Saw	80 · 6	13.2	6.2	1.9
15	289F-75 per cent ]	Mianchannu .	Saw	85 · 6	10.4	4.0	2.6
	4F-25 per cent						
16	289F	Khanewal	Saw	78.6	14.0	7.4	2.6
17	De i-15 per cent	Lyallpur	Saw	80.8	14.4	4.8	2 · 1
	4F—85 per cent			000			
18	4F	Mianchannu .	Saw	90.0	6.4	3.6	1.3
19	4F	Khanewal	Saw	88.0	5.2	6.8	2 . 2
	289F	Sargodha	Saw	82.0	10.8	7.2	$\overline{2} \cdot \overline{0}$
20	289F	Mianchannu .	Saw	87.2	6.8	6.0	$2 \cdot 2$
21		기가 되었다. 이번 생각이 살면 하다 중에 다		88.2	6.6	5.2	2.8
22	4F		Saw		The second second second	7.0	6.2
23	289F	1	Roller	67.4	25.6	1.0	0.2

cleanliness especially from dust and sand, cannot be sufficiently stressed, as these impurities are mainly responsible for the high ash content of chemical cotton prepared from linters.

Effect of variety and gin. Taking 1938-39 samples first it will be seen that the results of mechanical analysis vary very considerably even for the same variety. For example, three samples of 285F differ to the extent of about 20 per cent fibre content and four samples of 289F show even a still greater variation. Most probably these differences are due to the degree of care and attention paid to delinting at the various stations.

The type of gin seems to have a very pronounced effect on the quality of linters. For example, Nos. 7 and 8, both belong to 289F and are both delinted at the same station; yet they gave very different results, showing that saw gin is preferable to roller gin, so far as the quality of linters removed subsequently is concerned. This conclusion is supported by the results pertaining to Nos. 12 and 15 which are mixtures of the same variety of seed in the same proportions, but were ginned in roller and saw gin respectively. We may, therefore, conclude on the basis of these results that the quality of linters is better from a saw-ginned than from roller-ginned seed.

Turning to the 1939-40 samples, it will be noticed that the same type of seed has given very nearly the same quality of linters regardless of the station at which it was delinted. Small variations are of course unavoidable, but on the whole, the results of the eight samples of 4F, six samples of 289F and three samples of 285F are much more concordant and uniform than those observed for the 1938-39 samples. Comparison of the different varieties of seed shows that on the average 4F seed gave linters with slightly higher fibre content and slightly lower trash content than those from 289F or 285F seed among which there was nothing much to choose in this respect. The invisible loss was also lower, on the average, for the linters from the 4F seed than for the other two types of seeds, but the average ash content was lowest for the linters from the 285F seed.

As regards the effect of the type of gin, there is only one instance in which both roller and saw gins had been used with the same seed, viz. 289F. The difference of nearly 20 per cent in the fibre content of the two samples—Nos. 21 and 23—supports the conclusion drawn from the linters of the previous season that saw-ginned seed gives a better quality of linters than the roller-ginned seed.

The types of machines used for delinting the seed were generally speaking either 'Continental' or 'Carvers', the former being somewhat more common than the latter. In one case we came across 'Verner' type of machine while at another place locally made delinting machine seems to have given quite satisfactory results.

Results of the chemical analysis of Indian linters of two seasons are given below in Tables II (a) and II (b):

Table II (a)
Results of chemical analysis of Indian linters
1938-39 season

		·		
Serial No.	Chemical cotton per cent	Alpha- cellulose in chemical cotton per cent	Dry alpha- cellulose in air- dry linters per cent	Ash per cent
1	74.0	98.0	67-1	0.08
2	76.0	99.0	69.6	0.07
3	77.0	98.0	69-8	$0 \cdot 12$
4	66.0	97.0	59.2	0.14
5	58.0	85.0	45.6	0.15
6	78.0	99.0	71.4	$0 \cdot 29$
7	74.0	98.0	67-1	0.37
7 8 9	60.0	96.0	53.3	0.41
9	46.0	90.0	38 · 3	0.24
10	75.0	98.0	68.0	0.09
11	68.0	95.0	59.7	0.41
12	49.0	91.0	41.2	2.12
13	62.0	98.0	56.2	0.28
14	58.0	94.0	50.4	0.13
15	68.0	96.0	60-4	0.16

(b) Discussion of the results of chemical analysis

The results of the chemical analysis are given in Tables II (a) and II (b) in terms of the percentages of chemical cotton in linters, of alpha-cellulose in chemical cotton, of dry alphacellulose in air-dry linters and of the ash percentage of chemical cotton; these are dealt with individually below.

Chemical cotton. In case of the 1938-39 samples [Table II (a)] the variation in the percentages of chemical cotton is roughly of the same magnitude as shown by the fibre content [Table I(a)]. Similarly, the linters of the 1939-40 season [Table II (b)] show a much smaller variation in their chemical cotton percentages, which is roughly of the same order as the variation shown by the fibre content of these samples [Table I (b)]. This correspondence between the results of two treatments indicates the likelihood of a definite relationship between the two series. It must, however, be remembered that it is not the chemical cotton, but the alpha-cellulose which is regarded as a standard or criterion of quality by the cellulose industry and we shall therefore proceed to consider the results pertaining to alpha-cellulose.

Alpha-cellulose in chemical cotton. The alphacellulose content of the chemical cotton obtained from linters of the 1938-39 season was found

Table II (b)

Results of chemical analysis of Indian linters
1)39-40 season

			2.85	
Serial No.	Chemical cotton per cent	Alpha- cellulose in chemical cotton per cent	Dry alpha- cellulose in air-dry linters per cent	Ash per cent
1 2 3A 3B 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22	76·4 78·0 74·2 82·0 83·4 84·2 81·4 80·2 74·0 73·6 80·0 79·2 80·0 76·6 80·2 75·6 79·6 86·2 83·0 77·2 81.8 83·2	98·2 97·7 98·5 98·4 98·1 98·6 98·3 98·8 98·5 97·2 98·4 96·5 97·9 98·1 97·3 96·9 97·8 98·0 97·5 98·0 98·3	69·4 70·5 67·6 74·6 75·7 76·8 74·0 73·3 67·4 66·2 72·8 70·7 72·4 66·2 68·9 71·5 68·4 72·5 78·9 74·8 70·0 74·3 75·7	0·07 0·06 0·07 0·05 0·05 0·07 0·07 0·07 0·06 0·13 0·07 0·06 0·09 0·08 0·05 0·06 0·08 0·05 0·06
23	62.0	98.1	56.3	$0 \cdot 15$

to vary from 85 to 99 per cent. A variation of this magnitude is undesirable from the commercial point of view as the cotton generally contains a very small quantity of hemi-cellulose which do not ordinarily exceed 2 per cent. The high percentage of hemi-cellulose in some of the 1938-39 linters can only be attributed to the presence of foreign matter in the form of leaf or seed rests which must have persisted even up to the stage of the chemical cotton.

If we compare Tables I (a) and II (b) with a view to establishing a relationship, if any, between the alpha-cellulose content of the chemical cotton and the trash percentage present in the original samples, it will be found that up to a trash content of 17.5 per cent the linters yield a chemical cotton with an alpha-cellulose content of not less than 98 per cent which is the permissible minimum for rayon manufacture. But linters having a trash content of 17.5 - 32.5 per cent yielded chemical cotton with alpha-cellul se content varying from 94 to 98 per cent while the chemical cotton prepared from linters having a still higher trash content, namely from 32.5 to 47.5 per cent possessed alpha-cellulose content varying from 85 to 91 per cent. The only exception to this grouping is No. 13 which in spite of having a trash content of more than 17.5 per

cent yielded chemical cotton with 98 per cent alpha-cellulose. This, however, was due to the fact that the sample contained a considerable quantity of seed, which was removed before it was subjected to the chemical treatment.

Turning now to 1939-40 samples we find again that, on the whole, the linters are much superior to those of the previous season as two-thirds of these yielded chemical cotton with alpha-cellulose content up to the industrial standard of 98 per cent or above. Even in the remaining one-third the alpha-cellulose content is not very low, the lowest value being 96 5 per cent.

It is, however, noteworthy that unlike the 1938-39 samples, those of 1939-40 do not show direct relation between trash percentage and alphacellulose content while it may be stated in general that linters having low trash percentage will yield a chemical cotton with a high alpha-cellulose content. It must be remembered that it is not always the quantity but also the nature of the impurities present in raw linters which will finally determine the alpha-cellulose content of the chemical cotton from it. Thus, whereas a fairly high trash content in the form of leaf, stalk bits, etc., would be removed in the chemical process and may not therefore affect appreciably the alpha-cellulose content of the chemical cotton, a relatively low trash content in the form of undeveloped or crushed seed may persist in the chemical treatment, and may lower the alpha-cellulose content beyond the industrially permissible limit.

Dry alpha-cellulose in air-dry linters. The dry alpha-cellulose content of the air-dry linters is the real indicator of the quality of linters from the industrial point of view. This has been calculated for both the seasons and the values are given in Tables II (a) and II (b).

Ash content of chemical cotton. As may be seen from Table II (a), the ash content of the chemical cotton obtained from linters of 1938-39 season varied from 0·07—0·41 per cent with the exception of No. 12 of which the ash content was found to be as high as 2·12 per cent. While discussing ash content of raw linters, the abnormally high values were attributed to the presence of dust particles present in the samples. This explanation is actually corroborated by the following experiment.

Chemical cotton No. 12 which gave an abnormally high ash content of 2·12 per cent was shaken for half an hour in water over a fine sieve for removing small particles of dust. By this simple treatment the ash content came down to 0·59 per cent, i.e. nearly one-fourth of the original value. Three further similar treatments brought down the ash content of the same sample to 0·35 per cent proving definitely that the high

percentage of ash was due to the presence of dust particles embedded in the linters which could not be removed by the ordinary treatment given to all the samples but required a special treatment.

The tolerance for ash content is 0.12 per cent in case of industrial chemical cotton, while the ash content of the chemical cotton obtained from a number of samples was found to be higher than 0.12 per cent [Table II (a)]. As shown above, it can be brought down by suitable adjustments in processing in the form of either a preliminary mechanical cleaning or a series of rifflers preceding the final washing. Such treatment, however, would add to the cost of manufacture of chemical cotton, and, therefore, the best course would be to improve delinting so as to avoid admixture of dust with linters.

The ash content of the chemical cotton of 1939-40 linters is found to be satisfactory in all cases except only two, namely Nos. 9 and 23.

While the percentage of trash gives no indication as to the amount of dust present in the sample, the ash content of raw linters and that of chemical cotton prepared from it are associated with one another. In view of this observation the ash content of raw linters may advantageously be introduced as a factor in the grading of linters.

Wax. As some samples of linters (of the 1938-39 season) appeared to contain more oil or fat than the rest, it was decided to treat three selected

samples with benzine in a Soxhlet apparatus for six hours for fat extraction. The results are stated below:

Serial No.				Percentage of	
5 .	•			benzene extract	
9 .		•	•	0.64	

In each case the fat and wax content is somewhat higher than the highest found in Indian cottons. Its presence, however, is not harmful as the fat would be saponified during the alkali treatment. To test this point the wax content of the chemical cotton of No. 9 which gave the highest wax content in raw state was determined and found to be 0.12 per cent which is below the tolerance limit of 0.34 per cent, showing that most of the original fat and wax in the sample had been removed by the normal chemical treatment. The position, however, would be quite different if a sample were contaminated with mineral oil, which cannot be saponified by the alkali treatment, and would therefore remain in the chemical cotton possibly rendering it unsuitable for industrial use. Contamination with mineral oils must therefore be avoided.

Surface grading. The results of surface grading of linters, which it must be pointed out, have been tried for the first time in India are given in Tables III (a) and III (b).

Table III (a)
Results of the surface grading of Indian linters 1938-39 season

Serial No.	Matching U. S. Grade No. in		Colour		Foreign m		
	Character Colour	Colour		Leaf	Stalk	Seed	Remarks
1 2 3 4 5 5 6 7 8 9 10 11 12 13	5 2 2 (Between 4 4 (Between 1 2 4 (4—5) 2 (Between 3 (Between 4	4 and 2) 2 4 and 4)	Yellowish white Greyish white	Some Fair amount of small brown leaf Fairly large quantity of large brown Leaf Fair amount of large leaf Some brown and some black leaf Fair amount of black peppery leaf Some black and brown leaf Large amount Some peppery leaf Some black peppery leaf Fair amount of brown leaf Some brown leaf Some black peppery	Some	Small quantity of broken seed Plenty of cut seed Small quantity of seed Large amount of seed Large amount of crushed seed	Very small quantity of cotton fibre was seen distributed throughout the sample  The foreign matter was sticking to and uniformly distributed throughout the sample  Contained a small quantity of cotton fibre
14 15{	3 A 1 B (Between 2	1 ]	Greyish white	Fairly large quantity	Fairly large quantity Some		The red leaf which the sample contained appears to be foreign in its origin This sample was a heterogenous mixture of 3 possibly 4 grades

# Table III (b)

Results of surface grading of Indian linters 1939-40 season

Serial No.	Matching U. S. Grade No. in		Colour	Foreign matter			Remarks
	Character	Colour	OGG	Leaf	Stalk	Seed	
1	3		Greyish white	Some small brown and peppery leaf	Some	••••	It also contained boll-rests
2	23		,, ,,	,, ,, ,,	Few	Undeveloped green seed	
3A	4		Greyish	Small	Small	Fair amount of undeveloped seed	It also contained some boll husks
		3		Small black peppery		Few	
${3B\atop II}$	3	1		leaf	••••	Large amount .	Also some black seeds (Egyptian?
	1 2	30 S S S S	Greyish white	Small		Some whole seed	
4 5	3		,, ,,	Fair quantity of small black leaf		Small quantity	
6	3	•	Dirty yellow	Fair quantity of brown leaf			Some cotton fibres distribute throughout
7	3	••	Very dirty yellow	Large quantity		Some crushed seed	
8	6-7	6—7	Dirty brown	• • • • • • • • • • • • • • • • • • •	• • • • • • • • • • • • • • • • • • • •		[1] - 15를 보고 있습니다. 그 10 전 - 12 전하다고 15 2일 (15 15 15 15 15 15 15 15 15 15 15 15 15 1
9	2		Greyish white	Fair amount	••••		Also boll-rests
10	3	3		Some			
11	2		Greyish white	Slight		Fair quantity .	
12	1		Whiter than U. S. Grade No. 1	Some		Small quantity of undeveloped seed	
13	2		Whiter than U.S. Grade No. 2	Slight		Large quantity of whole seed	Some of the seeds were in clusters
14	2		Greyish white	Large quantity of peppery leaf	••••	Some crushed seed	
15	1—2	1—2		Some leaf, also peppery		Fairly large quantity of crushed seed	
16	2—3	2—3		Black peppery leaf		Slight crushed seed	
1 <b>7</b>	3		Greyish white	Fairly large amount of brown and peppery leaf		Some crushed seed	
18	1		Whiter than U. S. Grade No. 1	Fair amount of dark brown leaf		Some unde- veloped seed	
19	2		Greyish white	Some dark brown and black peppery leaf		Slight	
20	2	••	Whiter than U. S. Grade No. 2	Small quantity of brown and black peppery leaf		Some whole seed	
21	1	1		Small		Small quantity of whole crushed seed	1
22	2 .		Whiter than U. S. Grade No. 2	Small quantity of black peppery leaf		Very small quantity of whole and crushed seed	
23	3	ļ	Whiter than U. S. Grade No. 3	Plenty	Plenty	Plenty cut and whole	

(c) Discussion of the results of surface grading

In connection with this grading it is important to bear in mind two points. In the first place it to take into account staple, customary foreign matter, colour and character indicating the grade of a sample. In the present work, however, only the staple and character of Indian linters have contributed towards the fixing of the grade, the other two factors, viz. the colour and foreign matter having been noted down separately. Secondly, no effort has been made to give a final grade to any sample. Only in case of character a definite grade number is given, but the final grade must emerge out of it after the other factors especially colour and amount and type of foreign matter are given due consideration. In the case of colour, it is not yet established to what extent 'dirty yellow', 'dirty brown' or 'greyish white', etc., should influence the grade number while as regards foreign matter, it will be seen that this important factor has been simply noted down as observed. In the present state of affairs this procedure was inevitable, but when Indian standards of grades are prepared, these factors will have to be taken into account, and then it would be possible to fix closely the grade of a sample by comparison against the standards. Even with the limitations noted above, the results of surface grading are very interesting and are discussed below.

Staple and character. We note from Table III (a) that five samples out of 15 matched the American grade No. 2, one was slightly better, while of the remaining eight were inferior, matching grade Nos. 3, 4 and 5. One sample—No. 15—was peculiar in the sense that it was a clear mixture of two types of linters, one of which was superior to the other. None of the 15 samples matched the American grade No. 1, while none of them was below No. 5 in grade. Thus, the limits, in terms of the American grades, for these 15 samples were set by No. 2 and No. 5, roughly one-third matching No. 2 and the remaining being of inferior grades. Turning to the 1939-40 samples we find that three out of the 24 samples were of such superior quality as to match the American grade No. 1 in character, one was slightly lower (between 1 and 2), 8 samples matched grade No. 2, two were slightly lower (between 2 and 3), seven samples matched grade No. 3, while only two samples were below grade No. 3. Among the latter only one sample was of real inferior quality lying between grade Nos. 6 and 7, while the other matched grade No. 4. Here again one sample—No. 3B—was found to be a mixture of two types, one of which was definitely superior to the other. Thus, if we ignore only two samples Nos. 3A and 8 the limits for these samples are set

by grade Nos. 1 and 3, the majority of them matching either No. 2 or No. 3. These results bring out clearly the improvement in the grade of the 1939-40 samples as compared with those of the earlier season, of course, with respect to staple and character.

Colour. The colour of Indian linters was found in most cases to be different from that of the American standard which were 'reddish brown', the colour being practically absent in standard No. 1 and successively increasing in intensity towards the last standard No. 7. On the other hand, some of the Indian linters were white, whiter than even American standard No. 1, others were greyish, white to grey, while yet others were more or less brownish in colour.

It is interesting to note that as a rule the 4F seed gave linters which were greyish white in colour in general and white or grey in a few cases. In case of mixtures wherever the 4F seed predominates, the greyish white colour was to be noticed. On the other hand the linters from the 289F seed showed in most cases a distinct brownish colour though in a few cases it was white or dirty brown. On the whole the colour of the linters obtained from the 289F seed resembled the colour of the American linters, while the linters from the 285F seed resembled those of 289F seed, though the former showed a yellowish tinge which lent a rather dirty appearance to the linters.

Foreign matter. This is sub-divided into three heads—leaf, stalk and seed—and the quantity visually observed in each case is also set down in terms of 'fair', 'large', 'fairly large' 'small', 'slight', etc.

It is interesting to note in this connection the relation existing between the kind and quantity of foreign matter present in the raw linters and the alpha-cellulose content of the chemical cotton obtained from them. The general conclusions which are applicable to linters of both the seasons, are given below.

- (a) So long as the quantity of leaf or stalk was 'small' or in 'fair amount' and the sample did not contain either crushed or undeveloped seed in an appreciable quantity the chemical cotton obtained from it gave an alpha-cellulose content of at least 98 per cent.
- (b) If, however, the sample of linters contained 'large' of 'fairly large' quantity of leaf or an appreciable quantity of undeveloped seed or even a small quantity of crushed or whole seed which is not removed by mechanical means, the chemical cotton obtained from it gave an alpha-cellulose content of less than 98 per cent. Thus, the presence in linters of seed, particularly crushed seed is very detrimental to the quality of the

TABLE IV

Comparison of some results of mechanical analysis and chemical analysis, 1938-39 season

Serial No.	Fibres mechanically analysed— Per cent A	Chemical cotton chemically analysed— Per cent B	Dry alpha-cellu- lose in air-dry linters— Per cent C	Difference (A—B)	Difference (A—C)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15	$\begin{array}{c} 62 \cdot 5 \\ 75 \cdot 0 \\ 72 \cdot 5 \\ 65 \cdot 0 \\ 52 \cdot 5 \\ 80 \cdot 0 \\ 75 \cdot 0 \\ 57 \cdot 5 \\ 37 \cdot 5 \\ 75 \cdot 0 \\ 67 \cdot 5 \\ 45 \cdot 0 \\ 60 \cdot 0 \\ 57 \cdot 5 \\ 70 \cdot 0 \\ \end{array}$	$74 \cdot 0$ $76 \cdot 0$ $77 \cdot 0$ $66 \cdot 0$ $58 \cdot 0$ $78 \cdot 0$ $74 \cdot 0$ $60 \cdot 0$ $46 \cdot 0$ $75 \cdot 0$ $68 \cdot 0$ $49 \cdot 0$ $62 \cdot 0$ $58 \cdot 0$ $68 \cdot 0$	$67 \cdot 1$ $69 \cdot 6$ $69 \cdot 8$ $59 \cdot 2$ $45 \cdot 6$ $71 \cdot 4$ $67 \cdot 1$ $53 \cdot 3$ $38 \cdot 3$ $68 \cdot 0$ $59 \cdot 7$ $41 \cdot 2$ $56 \cdot 2$ $50 \cdot 4$ $60 \cdot 4$	$-11 \cdot 5$ $-1 \cdot 0$ $-4 \cdot 5$ $-1 \cdot 0$ $-5 \cdot 5$ $2 \cdot 0$ $1 \cdot 0$ $-2 \cdot 5$ $-8 \cdot 5$ $0 \cdot 0$ $-0 \cdot 5$ $-4 \cdot 0$ $-2 \cdot 0$ $-0 \cdot 4$ $2 \cdot 0$	$\begin{array}{c} -4\cdot 6 \\ 5\cdot 4 \\ 2\cdot 7 \\ 5\cdot 8 \\ 6\cdot 9 \\ 8\cdot 6 \\ 7\cdot 9 \\ 4\cdot 2 \\ -0\cdot 8 \\ 7\cdot 0 \\ 7\cdot 8 \\ 3\cdot 8 \\ 3\cdot 8 \\ 3\cdot 8 \\ 7\cdot 1 \\ 9\cdot 6 \end{array}$
Total	952.5	989 · 0	877 · 3	-36⋅5	75 · 2
Mean Cceff. of va- riation	63.5 per cent	65.9 per cent	58.5 per cent	2·4 156·9 per cent	$5 \cdot 01$ $75 \cdot 0$ per cent

chemical cotton obtained from them, while the presence of leaf in linters is relatively less deleterious.

# III. Comparison of the results of mechanical analyses

A comparison of the values given in columns A and B in Table IV shows that in most cases the yield of chemical cetton is actually higher than that of the clean fibre as shown by the values in column D. This apparent discrepancy is due to two factors: (1) some of the short fibres present in a sample are blown off as invisible loss in the Shirley Analyser, while in the chemical treatment they contribute towards the yield of the chemical cotton and (2) in the Shirley Analyser some neps and motes are lost as trash while these too give their quota in the chemical treatment. Since the alpha-cellulose content, in which the useless hemi-celluloses are left out of consideration is very important, its values are reproduced in column C while column E gives the difference between it and the fibre content.

The coefficients of variation of the differences have been worked out in each case. It will be

seen that the coefficient of variation of the differences between the fibre content and chemical cotton is more than double of that obtained for the differences between fibre content and alphacellulose, showing that alpha-cellulose bears a closer relation to the fibre content than chemical cotton. Nevertheless its high value of 75 per cent should serve as a warning against accepting the fibre-content of mechanical analysis as a true index of the alpha-content of a sample of linters.

In view of the fact that the invisible loss sustained in the Shirley Analyser is almost wholly of short fibres or fuzz which, as explained above, make their contribution in the chemical treatment, an attempt was made to find a relationship by adding the invisible loss to the quantity of fibres actually separated by the Shirley Analyser. The results obtained are given in Table V.

It is very interesting to note that the coefficient of variation of the differences between the fibre content and the alpha-cellulose, which was 75 per cent (Table IV), has, by the inclusion of the invisible loss, come down to  $13 \cdot 1$  per cent for the 1938-39 season samples. Similarly for the 1939-40 samples the coefficient of variation of the differences between the total fibre content and the alpha-cellulose content works out at  $10 \cdot 1$  per cent

Table V

Differences between the total fibre and alpha-cellulose contents in linter samples

Serial No.	$\begin{array}{c} {\rm Fibre} \\ {\rm per \ cent} \end{array}$	$\begin{array}{ccc} \text{Invisible} \\ \text{loss per cent} \\ \end{array}$	Total fibre per cent $F+f$	Dry alpha-cellulose in air-dry linters α	Difference— Total fibre minus alpha- cellulose $F+f-\alpha$
		(	1938-39		The state of the s
1 2 3 4 5 6 6 7 8 9 10 11 12 13 14 15	62·5 75·0 72·5 65·0 52·5 80·0 75·0 57·5 37·5 75·0 60·0 57·5 45·0 60·0 57·5 70·0	$\begin{array}{c} 22 \cdot 5 \\ 10 \cdot 0 \\ 10 \cdot 0 \\ 10 \cdot 0 \\ 12 \cdot 5 \\ 10 \cdot 0 \\ 7 \cdot 5 \\ 10 \cdot 0 \\ 10 \cdot 0 \\ 15 \cdot 0 \\ 10 \cdot 0 \\ 12 \cdot 5 \\ 10 \cdot 0 \\ 12 \cdot 5 \\ 10 \cdot 0 \\ 10 \cdot 0 \\ 10 \cdot 0 \\ \end{array}$	85·0 82·5 77·5 62·5 87·5 85·0 67·5 52·5 85·0 80·0 72·5 67·5 80·0	67·1 69·6 69·8 59·2 45·6 71·4 67·1 53·3 38·3 68·0 59·7 41·2 56·2 50·4 60·4	17·9 15·4 12·7 18·3 16·9 16·1 17·9 14·2 14·2 17·0 20·3 13·8 16·3 17·1 19·6

Coefficient of variation:

13.1 per cent

			1939-40		
1	81.0	7.4	88-4	69.4	19.0
2	81 2	$6\cdot 2$	87 4	70.5	16.9
3A	$78 \cdot 2$	7.6	85 8	67.6	18.2
3B	83.0	7.2	$90 \cdot 2$	74.6	15.6
4	86.8	5.0	91.8	75.7	16-1
$\frac{4}{5}$	86.4	7 · 2	93 · 6	76.8	16.8
6	83 · 8	4.6	88 · 4	74.0	14.4
7 *	82.4	6.0	88 · 4	73.3	15.1
.8	$70 \cdot 6$	15.4	86.0	67.4	18.6
9	75.0	7.0	$82 \cdot 0$	66 · 2	15.8
10	82.0	8.6	90.0	72.8	17.8
11	83.0	6 · 4	89-4	70.7	18.7
12	82.0	7 · 4	89 · 4	72.4	17.0
13	76 4	5.6	82 · 0	$66 \cdot 2$	15 · 8
14	80.6	$6\cdot 2$	86 · 8	68.9	17.9
15	85 6	4.0	89.6	71.5	18-1
. 16	$78 \cdot 6$	7.4	86.0	68.4	17.6
17	80.8	4.8	85.6	$72 \cdot 5$	13 · 1
18	90.0	3.6	93.6	78-9	14.7
19	88.0	6.8	$94 \cdot 8$	74.8	20 0
20	82.0	$7 \cdot 2$	89 · 2	70.0	19 · 2
21	$87 \cdot 2$	6.0	$93 \cdot 2$	74.3	18.9
22	88 · 2	5 · 2	93.4	75.9	17.5
23	$67 \cdot 4$	7.0	74.4	56.3	18.1

Coefficient of variation: 10.1 per cent

Total (39 readings)							658 6	
등 없었다. 그리아 보다 하기 하다 나 주었다.								
Mean (39 readings)							16.9	
김희물의 [2012년] 내용 경기 등에 다시 생각을 하는					• 17 B			
Coefficient of variation per cent	1.00						11.3	

which is even better than that obtained for 1938-39 samples. The mean value of this difference for the two seasons comes out to be 16.9.

The small values of the coefficient of variation and the general run of the data relating to linters of widely varying qualities belonging to two different seasons led us to suspect that a general relationship exists between the total fibre-content and the alpha-cellulose content of sample of Indian linters. In order to find this relationship a linear regression of the form y=a+bx, where y is the alpha-cellulose content  $(\alpha)$ , x is the total fibre content (F+f), and a and b are constants, was fitted to the 39 observations given in Table V, and its equation was found to be:

 $\alpha = F + f - K \dots (2)$ where K is a constant and has a value of 16.9.

This formula is not only very interesting but highly useful, as it enables one to predict, with a fair degree of accuracy, the alpha-cellulose content of a sample of Indian linters without actually performing the chemical test on it but merely from the results of mechanical analysis which is comparatively quicker and less elaborate. In order to judge the validity and limits of usefulness of this formula, the values of alpha-cellulose content of all the samples of linters of the two seasons were calculated and compared with the experimental values. Table VI gives the distribution of differences between the calculated and the predicted values.

TABLE VI

No. of value
8 12 10 2 Average 2.3 per cent 2 4 1

It will be seen that for 20 samples out of 39 the difference between the actual and the calculated values of the alpha-cellulose content is less than 2 per cent, while only in five cases it exceeds 5

per cent. The average difference between the actual and the calculated values for both seasons is only  $2\cdot 3$  per cent. This agreement must be regarded as very satisfactory and the formula may be used confidently for estimating the alpha-cellulose content of Indian linters.

# IV. COMPARISON OF THE SURFACE GRADING METHOD WITH MECHANICAL ANALYSIS

The method generally adopted in America for grading linters takes into account the following four points (1) staple, (2) foreign matter, (3) colour and (4) character. The significance of each of the factors may be briefly explained as follows:

Staple. The exact significance of this term is somewhat different from what is generally understood in the case of cotton. In case of linters it refers to the aggregate impression or estimation of the different blends of long and short fibres present in a sample, and is determined chiefly by the relative proportion of each class of fibre.

Foreign matter. It generally consists of stalks, leaves, motes, hull particles, dirt and any other matter adhering to the linters. In preparing the standards, allowance is made for specified quantities of foreign matter in each grade. All samples having more foreign matter than that specified in a standard are classed 'off grade'.

Colour. Olive and buff are the colours referred to in American official grades, but generally those who deal in linters call them 'green' and 'cream'.

Character. This property is rather difficult to define in terms of physical attributes. It refers to the section of the cotton belt, or the area in which the seed cotton, from which the linters are obtained, is grown.

In United States of America linters have been standardized in seven grades. The delinting process may be carried out in two ways. The seed may be delinted in one single operation removing the long as well as the short fibres together, the linters thus obtained being designated 'Mill-run'. Alternatively, the seed may be delinted in two successive operations, the first one removing mostly the long hairs called 'first cut' and the second one removing the remaining fibres on the seed, called 'second cut'. The 'first cut' generally gives grades Nos. 1, 2 and 3, while the 'second cut' delivers grades Nos. 5, 6 and 7. The proportion of the first cut to the second cut is roughly 1: 4 by weight. Very often the seed is delinted at once and the 'millrun' thus obtained is generally classified in grades Nos. 3, 4 and 5.

First cuts: Cuts ranging from 20 to 50 lb. per ton of seed have generally become known as first cuts or first cut linters.

Mill runs: Cuts ranging from about 35 to 100 lb. or more per ton of seed are known as mill runs or mill run linters.

Second cuts: After a first cutting, especially if not over 50 lb. per ton of seed have been removed in the first delinting, the seeds are frequently passed through the delinting machine a second time. The linters thus recovered are known as second cuts. The total quantity of linters cut ranges from about 30 lb. to as high as 200 lb.

per ton of seed. In India the processes of recovering the linters have not yet become standardized, while no grades are available against which the samples offered in the market may be matched and valued. It is hoped that this investigation, in which a new method of grading linters has been worked out, will lead to the preparation of commercial grades. It will be remembered that 39 samples of Indian linters belonging to two seasons were subjected to mechanical and chemical treatments, besides being graded as best as possible under the circumstances by the surface grading method, and the results of the two methods have been Since this method is offered to compared. serve as a basis for the preparation of standard grades suitable for Indian linters, it was felt that it would be instructive to examine the American grades by this method. For this purpose, American standard grades of linters which were obtained from U. S. A., were subjected to mechanical and chemical treatments and the results obtained are given in Tables VII

Table VII (a)
Mechanical analysis

(a) and VII (b).  $\cdot$ 

	Per cent		L CL COLLE	Per cent	Per cent dry alpha- cellulose— calculated
No.	fibre	trash	invisible loss	ash	(F+f-K) = $\alpha$ where
			1088	9	$K=16 \cdot 9$
1	93.8	3.2	3.0	1.04	80.0
2	88.8	4.6	6.6	1.09	78.6
3	84.8	6.8	9.4	1.11	77.4
4	77.0	9.6	13.4	1.12	73.6
5	$69 \cdot 2$	11.0	19.8	1.16	$72 \cdot 2$
6	64.0	13.8	22 · 2	1.42	69.4
7	60.8	15.6	23 · 6	1.62	67-6

Table VII (b)
Chemical analysis

Grade No.	Per cent chemical cotton	Per cent alpha- cellulose in chemical cotton	Per cent ash	(Experimental) Per cent dry alphacellulose in air dry linters
1	87 · 8	$99 \cdot 5$	0.04	80.8
2	85 3	99-1	0.05	$78 \cdot 2$
2	$84 \cdot 2$	98.7	0.07	$76 \cdot 9$
4	81 · 8	$98 \cdot 9$	0.07	$74 \cdot 8$
5	78.4	$99 \cdot 1$	0.07	$71 \cdot 9$
6	75.9	$96 \cdot 7$	0.07	$67 \cdot 9$
7	74.8	$96 \cdot 6$	0.08	66.8
				<u> </u>

The most interesting feature of Tables VII (a) and VII (b) is the very close agreement which has been found to exist between the calculated and the experimental values of the dry alphacellulose content as given in the last columns of the two tables. This establishes the validity and indicates the usefulness of the formula  $F+f-K=\alpha$ , which enables us to grade linters with accuracy and speed. A new method of grading linters, based on this formula, is suggested below:

A representative sample weighing about 100 gm. of the linters, which are to be graded, should be passed through the Shirley Analyser, and the trash should be passed again and again until a measurable quantity of fibres cannot be recovered from it. The separated fibres and the trash should be weighed carefully, and the invisible loss should be ascertained by deducting these two from the original weight of the sample. By substituting the actual values for F (separated fibres) and f (the invisible loss) in the formula  $F+f-K=\alpha$ , K being a constant equal to 16.9, the dry alpha-cellulose content of the sample should be obtained. Table VIII should then be used to decide the grade in which the sample should be placed.

TABLE VIII

In case of per lose (dry) i	cent α n air nters	cellu- dry		classe de <b>No.</b>	
80 and abo	ve			1	
76 and 80				2	
72 and 76				3	
68 and 72				4	
64 and 68				5	
60 and 64				6	
56 and 60				7	

[XV

In case the alpha-cellulose content is less than 56 per cent, the sample should be regarded 'off grade'. It will be noticed that the lower limit of α-cellulose suggested above is 56 per cent as against 66 per cent found for the American standard grades. It should, however, be noted, in this connection, that whereas the mechanical analysis tests have been made on the commercial samples of Indian linters obtained from the delinting factories, these tests have been carried out on the American standard grades which are carefully prepared by the U.S. A. Department of Agriculture. Therefore, the two sets of samples and their results are not strictly comparable, and the results of tests on American standard grades can only be taken as a rough guide for fixing tentative standards for Indian linters. These results would have been comparable if similar tests could have been carried out on commercial samples of American linters, which would have revealed how far, in actual practice, they deviate from their standard grades. This was, however, not possible owing to the extraordinary conditions prevailing at present. Until such tests have been carried out on American as well as a larger number of commercial Indian linters, the standards suggested here should be regarded as tentative, which should serve as a working basis, but which should be modified, if necessary, in the light of further experience.

Since it may not be possible in each case to pass a sample of linters offered in the market through the Shirley Analyser, it is desirable to prepare, with the help of this method, boxes of standard grades from typical Indian linters, so that a large number of samples may be matched against them, as is done in the case of

cotton.

We shall now see to what extent this method of grading samples of linters would give the purchaser useful information regarding the quality of the material. According to the American method of grading, each grade gives the purchaser some information about staple, foreign matter, colour and character. The last two items, however, do not appear to be so important as the first two items, especially when the linters are to be transformed into chemical cotton. Whether a sample of linters looks 'grey' or 'brown' and whether it has been obtained from a Sind-American or Punjab-American seed would make practically no difference to the quality of cellulose obtained from it. Thus, leaving these two items, i.e. colour and character, out of account in arriving at a method for grading linters, we find that the staple and the amount of foreign matter in a sample are the most important factors in determining its grade.

The method proposed above must therefore satisfy the purchaser regarding staple and foreign matter present in each grade. If, instead of giving information regarding the quantity of foreign matter present in a sample, it were possible to give the exact amount of alpha-cellulose available in that sample, it would be a definite improvement over the old method. The staple also plays an important role and should be accounted for in the proposed method of grading, the alpha-cellulose content being independent of the staple. With a view to finding out a simple and reliable method of expressing the staple of a sample of linters, it was essential to know the staple of the standard American grades of linters, although this work was labourious and time-consuming. The group-length distribution in percentage by weight of the standard grades is given in Table IX.

TABLE IX

Distribution of group-lengths in U. S. grades (1939-40)

Group length	lst grade	2nd grade	3rd grade	4th grade	5th grade	6th grade	7th grade
/16 in. & less /8 in /8 in. /8 in /8 in /8 in /8 in. /8 in. /8 in. /8 in. /8 i	1:4 16:3 11:5 8:1 10:7 13:7 14:7 11:1 7:3 2:8 2:4	5-2 34·5 17·0 7·8 8·9 11·8 7·1 4·9 2·8	9 · 8 35 · 1 17 · 5 10 · 5 3 · 6 5 · 2 6 · 3 5 · 5 5 · 2  1 · 3	- 10·2 36·3 14·1 4·6 8·8 9·0 7·5 3·9 4·4 1·2	19·4 54·0 13·8 4·4 3·4 3·1 	22·3 55·6 16·1 4·5 1·5	23·0 65·6 8·7 0·7 0·9 1·1

A glance at Table IX shows that the percentage of the short group-lengths, particularly  $\frac{1}{16}$  in. and  $\frac{1}{8}$  in., goes on steadily increasing, while that of the remaining (long) group-lengths goes on generally decreasing to vanishing point, as the grade of the linters falls, although here and there a stray long fibre might be found even in the lower grades.

Furthermore it will be observed from Table IX that the seven grades may be resolved into three groups. The first group consists of grades No. 1 and 2, which possess a much larger percentage of long fibres ( $>\frac{1}{8}$  in.) than of the short ones ( $<\frac{1}{8}$  in.). Grades No. 3 and 4 form the second group in which the long and the short fibres are present in almost equal proportions. The third group is formed by grades No. 5, 6 and 7 wherein the proportion of short fibres is much larger than that of the long ones. It is interesting to remember in this connection that generally the 1st and 2nd grades are obtained from the 'first cut', 3rd and 4th grades from 'mill-run' and 5th, 6th and 7th grades from the 'second cut'.

This group-length determination of linters, though interesting, does not lead us any farther towards the solution of the problem of evolving a quick method of grading, because it requires so much time that it must be ruled out as a practical method. If, however, attention is centered on the shortest group length  $(\frac{1}{16})$  in.) only, on account of its being particularly re-presentative of the grades, and if it is assumed that the proportion of various group lengths in each grade is more or loss constant, as it should be, the solution of the problem draws nearer. This line of approach is supported by a comparison of the invisible loss in the Shirley Analyser and the percentage of the fibres possessing a group length of  $\frac{1}{16}$  in., which are practically identical. The small differences observed between the two are within sampling error, and we may therefore conclude that the invisible loss (per cent) in the mechanical analysis is equal to the percentage of  $\frac{1}{16}$  in. group length fibres in a sample of linters, this group length, on its part, being representative of the grade. In this way it is possible to indicate the staple of the sample with the help of the invisible loss without actually determining it. Thus, mechanical analysis will not only furnish information on the total amount of foreign matter present in a sample, but also on its staple.

The new method proposed for Indian linters is based on the tentative specifications given in Table X.

If we apply the criteria implied in this method of grading to the samples of Indian linters, which

TABLE X

Per cent dry alpha- cellulose in air-dry linters	Per cent invisible loss	Grade to be allotted	Per cent invisible loss in the 3 groups
80 and above	0—5	$\left[\begin{array}{c}1\\2\end{array}\right]$	0-8 'First
76 and 80	5—8		cut'
72 and 76	8—11	$\left\{\begin{array}{cc} 3 \\ 4 \end{array}\right\}$	8-16 'Mill-
68 and 72	11—16		run'
64 and 68	16—21	5	16 and more
60 and 64	21—23	6	'second
56 and 60	23 & more	7	cut'
Below 56		off	

formed the subject of investigation, we obtain results given in Table XI, which also shows the results of surface grading for purposes of comparison.

TABLE XI

Gomparison of the results of surface grading with those obtained by the proposed method

1938-39 Season

		Pre	oposed grad	ing
Serial No.	Surface grading— Grade No.	On ~ — basic (Calc.)	On f— -basis	Final grade
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15	\$\frac{1}{2}\$ \$\frac{1}{2}\$ \$\frac{2}{4}\$ \$\frac{4}{4}\$ \$\frac{1}{5}\$ \$\frac{2}{2}\$ \$\frac{4}{4}\$ \$\frac{5}{2}\$ \$\frac{2}{3}\$ \$\frac{4}{4}\$ \$\frac{5}{5}\$ \$\frac{2}{3}\$ \$\frac{1}{8}\$:2-4}	4 4 5 6 Off 4 4 Off 7 Off 7	6 3 3 4 3 2 3 3 4 3 4 3 4 3 4 3 4 3 3 4 3 3 4 3 3 4 3 3 3 3 4 3 3 4 3 3 4 3 3 4 3 3 4 3 3 4 3 3 4 3 3 4 3 3 4 3	5 4 4 5 Off 3 4 Off 5 Off 5 Off 4 5 Off 4 5 Off

N. B.—In fixing the final grade attention is paid both to the alpha-cellulose content and the invisible loss (per cent) of the sample. When the difference between the grades as indicated by the two methods is only one, the grade indicated by the alpha-cellulose content is taken as the final grade. Where the difference between the two grades is 2, the grade indicated by the alpha-cellulose content is raised or lowered, as the case may be, by one grade. Similarly, for larger differences. Where a sample is shown to be off-grade by the alpha-cellulose content, it is regarded as off-grade in the final evaluation, as the alpha-cellulose content is given the predominant place.

1939-40 Season

		Prop	osed grading	
Serial No.	Surface grading — Grade No.	On ∝ — basis (Calc.)	On f— basis	Final grade
1 2 3A 3B 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23	3 2—3 4 4 A 3 B 1 2 3 6—7 2 3 2 1 2 2 1—2 2 3 1 2 2 1—2 3 3 3 3	444 3 324345333543442233227	2 2 2 2 1 2 1 2 4 2 2 2 2 2 1 2 1 2 2 2 2	3 3 3 3 2 2 2 3 3 4 4 3 3 3 4 3 3 2 2 3 3 2 2 3 3 2 2 3 2 2 3 2 3

It will be seen that in the case of 1938-39 samples there is considerable difference in the grades obtained by the surface grading method and the proposed method. This is mainly due to the fact that the amount of the foreign matter cannot be properly estimated in the surface grading method, while it is accurately given by the chemical method. This difference is less conspicuous in 1939-40 season, as these linters contained less foreign matter than the 1938-39 samples. If the results of the surface grading method are compared with those obtained by consideration of the invisible less alone, it will be seen that the two agree fairly well in both seasons, showing that this method attaches greater importance to the staple of the sample. For final grading, however, both staple and foreign matter have to be considered jointly, with greater emphasis on the latter in view of the fact that it ndicates the quantity of the available cellulose. The fairly satisfactory agreement between the grades obtained by the two methods in case of linters of 1939-40 season, which were comparatively cleaner, shows that the surface grading method can be applied to linters, provided they are fairly clean and the grader lays proper emphasis on the quantity of foreign matter present in the linters in fixing the final grade.

It will be noticed from the invisible loss (per cent) of the linters that all samples of the 1938-39 season, with the exception of No. 1, which is a second cut, are 'mill-run'. In 1939-40 season all samples except No. 8, which is a 'mill-run', are 'first cut' as confirmed by the producers. It shows that there is nothing really wrong with the delinting industry in this country, except that here and there a few whole or crushed seeds find their way into the sample. A more serious fault, shown by this work, is the presence of large quantity of foreign matter in the linters, which, in actual practice, would detract from their grade and utility to the consuming industries. It is gratifying to note, in this connection, that the 1939-40 linters show a distinct improvement over those of the previous year in the matter of cleanliness and grade. Since the Indian linter industry is very young, it is reasonable to hope that this improvement noticed in 1939-40 will continue, and that within a short time the linters produced in India will be equal to those produced elsewhere in respect of grade and quality. In the meantime, effort should be made to remove this defect, which is responsible for placing 5 out of 15 samples of 1938-39 in the 'off grade' class, and for the lowering of the grade of several samples by one or even two grades in 1939-40

Turning to the choice of one of the two methods stated above, it is to be noted that the surface grading method is liable to subjective errors, and is therefore at best an approximation. proportion of the fibre to fuzz and the amount of various impurities present in the sample cannot be estimated with the eye as exactly as with a machine, the difficulty becoming greater in the lower grades. In the mechanical method, on the other hand, the percentage of fibres and trash is obtained by actual measurements. Once the results of the mechanical analysis are available. they may be substituted in the formula F+f-K $=\alpha$  to get the alpha-cellulose content of the sample, the staple being disclosed by the invisible loss. The mechanical method will naturally take more time than the surface grading method, but the former is preferable to the latter in view of the higher accuracy of the results obtained, where a Shirley Analyser is easily accessible.

It is true that in U.S.A., the largest producer of linters, the trade relies mainly on the surface

grading method. But there are indications to show that they are not satisfied with the existing state of affairs and have drawn up an extensive scheme of research on linters to be worked out in every detail, right from the effect of soil to chemical cleaning, in their newly-built regional research laboratories. Since the results of the investigation carried out here have pointed the way to a reliable and accurate method of grading linters which is capable of classifying them according to their alpha-cellulose content, we should take full advantage of these results and adopt this accurate method of grading linters whereever suitable testing facilities are availabe. however, such testing facilities are not readily accessible, then the trade may adopt the alternative method of comparing a given sample of linters with standard grades prepared by a recognized organization. It should, however, be borne in mind that in the final analysis and for accurate work only methods of chemical and mechanical testing are capable of giving reliable results. Such methods of grading would ensure the supply of linters of known and uniform quality, which would help in securing wide and permanent markets both at home and abroad, and would thus assist in building up the chemical cotton and the allied industries on sound lines.

#### V. ECONOMIC ASPECTS

We shall now discuss some of the economic aspects of the production of linters in India and the manufacture of chemical cotton from them. The first question, which we propose to consider, is to what extent Indian linters can be produced commercially for export purposes and for the manufacture of chemical cotton in the country. In this connection it is important to bear in mind that the production of linters in the Indian oil mills and delinting factories is a very recent development, and, therefore, the present output of a few thousand bales, which are offered every year for sale in the market, is not a true index of the potential supply of this material in the country. In order to form an approximate idea of the potential supply inquiries were made from the Departments of Agriculture of all the cotton growing provinces and States in India as to the quantity of seed available in each province which could be used for delinting purposes. From the information supplied it would appear that, at a very moderate estimate, the total quantity of fuzzy seed, which may be used for delinting, available in the country is nearly 9 lakhs of tons. In order to estimate the total quantity of linters recoverable from the seed it is necessary to know the yield of linters per ton of seed. Inquiries were addressed to several oil mills and factories, where delinting is being carried out at present,

and the figures kindly supplied by them are shown in column 8 of Table XII. Though the individual figures vary, as might be expected on account of differences in the type of seed, delinting machinery, etc., the average value calculated from their replies came to 45 lb. per ton, representing a yield of 2 per cent on the weight of the seed. On the basis of this average figure the potential annual supply of linters in India is estimated to be about 80,000 bales of 400 lb. each. In order to arrive at a more correct estimate, it would be necessary to conduct a survey with the two-fold object of finding out on the one hand the quantity of fuzzy seed grown in each area and on the other hand the amount of fuzz present on each type of We may mention here that attempts were also made to find out the total quantity of linters that are being actually produced in the country at present, but unfortunately no reliable data could be collected.

The potential supply of 80,000 bales of linters of 400 lb. each is not very large as compared with the American supply which in 1938-39 stood at 1,116,000 bales of 500 lb. each, being equivalent to nearly 1,400,000 bales of 400 lb. In making a comparison between the Indian and the American linters and in drawing conclusions regarding the future of the former industry, it is necessary to bear in mind certain salient points of difference. In the first place it must be remembered that the American cotton crop is, on the average, nearly  $2\frac{1}{2}$  times as large as the Indian cotton crop so that a very much larger quantity of seed is available in America for delinting purposes. Secondly, the bulk of the American seed is of the fuzzy type which can be delinted and which gives a fairly good yield of linters, while many varieties of the Indian seed are either very nearly naked or have a very small quantity of fuzz at their apical ends. The main types of fuzzy seeds available in India are P.A. 289F, 289F/43, 289F/K 25, Cambodia Co2, Upland Gadag, Sind-American, etc. while such types as Surti, L.S.S., Westerns, Broach desi, Bengals, etc. are of the non-fuzzy type. Thirdly, the production of linters in America has increased steadily owing to the demand for this material in U.S.A. and the world markets as it forms the raw material for numerous industries. When nearly 40 years ago the linters were first produced in the U.S. A., their total production amounted to only 100,000 bales. In a few years the production went up very considerably until in 1914-15 it stood at over 800,000 bales. Since then the production of linters in U. S. A. is shown in Table XIII which gives the production of cotton and linters in America from 1914-15 to 1938-39.

It is interesting to note that whereas the proportion of linters to cotton was only 5.2 per

Table XII
Particulars regarding Indian linters of 1939-40 season

Serial No.	Variety of seed	Place where delinted	Gin- Saw or Roller	First Cut, Second Cut or Mill	Price per md. of 82 2/7 lh.	Delinting machine used	Linters recovered per ton of seed	Match U.S Grad Cha- racter	Colour	Colour	Foreign matter
1	4F	Khanewal	Saw	First cut	Rs. 5 to 6 locally and loose	Conti- nental	45 lb. to 67 lb.	3		Greyish- white	Small brown and peppery leaf some stalk, also some
2	4 F	Mailsi	,,	,,	Rs. 5/8/- ,,	Carvers	<b>23</b>	2-3		,,	boll rests Some brown and peppery leaf, few stalks and some un-
3A	4 F	Vihari	,,	,,,	27 22	,,	,,	4		Greyish	developed green seed Small amount of leaf and stalk but fair amount of
											undeveloped seed; also some boll husks.
<b>3</b> B	289F	<b>15</b>	,,	,,	Rs. 6/- ,,	,,	,,	I 3 II 1	3	: II	Small amount of leaf and a few seeds.
4	20% Desi 80% P.A.	Okara .	,,	•••	•••	,,	45 lb.	2		Greyish white	seed, some black ones (Egyptian?). Small quantity of seed and some
5	50% Desi 50% P.A.		,,,	•	,	,,	,,	3		<b>33</b>	whole seed. Fair quantity of small leaf also small quantity of seed.
6	285F	Hyderabad Sind	Saw and Roller	First cut	Rs. 5/- f.o.r.	,,,	22	3		Dirty yellow	Fair quantity of brown leaf, some cotton fibres distributed through-
7	285F	3,3	Mixed	,,		,,,	,,	3		Very dirtv yellow	out the sample.  Large quantity of leaf and some crushed seed.
8	285F	Navsari		Mill run		,,	45 lb. to 56 lb.	6—7		Dirty brown	orașiică scoa.
9	4F	Burewala	Saw	First cut	Rs. 6/- to Rs. 8/-	Conti- nental	18-17 lb.	2		Greyish white	Fair amount of leaf, also some whole and crushed seed—some boll
10	289F	Sargodha	. ,,	,,	Rs. 5/10/-	"	60 lb.	3	3		rests also Some leaf and some whole and crushed
11	30% Lesi 70% PA. 289F	Okara	,,	,,	•••	Varner	40 lb.	2		Greyish white	seed Slight leaf and a fair
12	289F	Mian- channu	,,	<b>,,</b>		Conti- nental	55 lb.	* 1		Whiter than U.S. Grade	Slight leaf and a fair quantity of seed Some leaf and a small quantity of undeveloped seed Slight leaf and a
13	4F	Arafwala	,,	,,		**	25 to 28 lb.	2		Whiter than U.S. Grade No. 2	Slight leaf and a large quantity of whole seed, some of them in clusters
14	4F	Montgomery	,,		Rs. 8/- f.o.r.	,,	13 lb.	2		Greyish white	Large quantity of peppery leaf and some crushed seed
15	75% 289F 25% 4F	Mian- channu	,,	•		••	54 lb.	1-2	1-2		Some leaf and a fairly large quantity of crushed seed.
16	289F	Khanewal	,,	First cut	Rs. 7/4/- including pressing	27	27 lb.	2—3	2-3		Black peppery leaf and slight crushed seed
17	85% 4F 15% Desi	Lyallpur	- >1	,,	charges Rs. 8/-	,,	About 10 lb.	. 3		Greyish white	Fairly large amount of leaf and some
18	4F	Mian- channu	,,	,,	Rs. 7/-	,,	41 lb.	1		Whiter than U.S. Grade No. 1	crusehd seed Fair amount of leaf and some undeve- loped seed.
19	4F	Khanewal	,,	,,		29	17 Ib.	2		Greyish white	Some leaf and slight sand
20	289F	Sargodha	,,	,,	Rs. 4/8/-	Carvers	54 lb.	2		Whiter than U.S. Grade No. 2	Small quantity of seed and some
21	289F	Mian- channu	,,		$\left\{ \begin{array}{l} \text{1st and} \\ \text{2nd} \\ \text{quality} \\ \text{linters} \\ \text{at Rs. 6} \end{array} \right\}$	Country (self made) delint- ing	42 lb.	Ī	1	No. 2	whole seed Small quantity of leaf, as well as whole and crushed seed
22	4F	,,	,,	,,	,,	machine "	22 lb.	2		Whiter than U.S. Grade No. 2	Small quantity of leaf, and very small quantity of whole and crushed seed
23	289F	,,	Roller	"	3rd quality at Rs. 2/-	"	36 lb.	3		Whiter than U.S. Grade No. 3	and crushed seed Plenty of leaf, stalk and seed

TABLE XIII

Production of cotton and linters in America during 1914-15 to 1938-39

	(In 1,000 ba	les running)	
Season	Cotton	Linters	Percentage of linters on cotton production
1914-15 1919-20 1924-25 1929-30 1930-31 1931-32 1932-33 1933-34 1934-35 1935-36 1936-37 1937-38 1938-39 1939-40	15,906 11,326 13,639 14,548 13,756 16,629 12,710 12,664 9,472 10,420 12,141 18,252 11,623 11,928	832 595 858 1,038 824 876 741 801 805 876 1,127 1,471	5.2 5.3 6.3 7.1 6.0 5.3 5.9 6.3 8.4 9.2 8.0

cent in 1914-15 in 1938-39 it had risen to 9.6 per cent. The principal causes for this increase are: (a) steadily increasing demand, (b) improvement in methods of delinting and (c) the production, in later years, of 'mill run' and 'second cut' linters in addition to the first cut linters. We shall consider this last point in some detail. Most of the linters which are at present produced in India belong to the quality commonly known as 'first cut', while in America they belong to three qualities namely 'first cut', 'mill run' and 'second cut'. The definition of these three qualities according to U.S. Department of Agriculture\* staff has been given earlier in these pages.

The distribution of the American linters in these three qualities since 1933-37 is shown in Table XIV from which it will be seen that only about 20 per cent of the total quantity of linters in the U. S. A. are of the first cut quality, about 30 per cent are mill run, while about 50 per cent belong to the second cut quality.

TABLE XIV

Distribution of American linters In bales in the three qualities, First Cuts, Mill Runs and Second Cuts

Season	First Cuts	Mill Runs	Secnd Cuts	Total
1933-34	1,42,166	2,68,609	3,89,751	8,00,526
1934-35	1,56,772	2,57,934	3,90,377	8,05,083
1935-36	1,88,968	2,59,469	4,27,778	8,76,215
1936-37	2,74,944	3,18,310	5,33,619	11,26,873

<sup>\*</sup>U. S. Department of Agriculture Miscellaneous Publication No. 242, May 1936

The average yield of the mill run linters in U. S. A. is 3·2 per cent of the weight of the seed as against 1·6 per cent for the first cut, while the average yield of the second cut is 4·5 per cent. Thus, if the mill run and second cut linters are also recovered in India as is done in America, the total quantity of linters available in India would be very much greater than what it comes into the market at present. Taking all these factors into consideration, we feel confident in stating that when sufficient demand has been built up for Indian linters either for consumption within the country or for export purposes, a very nuch larger supply than is seen at present in the market will be available.

We shall next consider the question as to what proportion of this potential supply of linters would be available for purposes of export and what proportion would be available for the manufacture of chemical cotton in India. In the U.S.A. the linters are divided into seven grades according to the length, colour, character and amount of trash. The grade I linters, which have a good proportion of fibres which can be spun, are used for spinning low grade yarns and for the manufacture of mats and fleece-lined products. Grades 2, 3 and 4, which have larger proportions of short fibres, are used for making mattresses, pillows, cushions, felts, etc. while grades 5, 6 and  $\bar{7}$  which are sometimes referred to as the 'chemical grades' are generally used for the manufacture of chemical cotton. The distribution of linters in the different grades in U.S.A. will be seen from Table XV from which it will be noticed that the bulk of the linters fall in the grades 3-6, while comparatively smaller proportions conform to grades 1 and 2 on the one hand and grade 7 on the other.

We may mention here that in general the first cut linters give rise to grades 1 and 2, the mill run to grades 3 and 4 and the second cut to grades 5, 6 and 7. This classification, however, should not be regarded as rigid, as, for example, it is quite possible that a first cut sample may conform to grade 3 or a mill run sample to grade 5 depending upon such factors as colour, amount of trash, etc. In India the grading of linters on a systematic basis has not been carried out, and it is, therefore, not possible to give similar figures showing the distribution of Indian linters in the various grades. The surface grading which was done in the course of this work showed that in 1939-40 the bulk of the samples conformed to American grades 2, 3 and 4; but this cannot be taken as a true indication of the state of affairs in the future when in addition to the first cut linters both mill run and second cut linters will also be recovered from the Indian seeds. We may, theefore, assume that a large proportion of the Indian linters,

#### TABLE XV

#### Production of linters in U.S. A.—distribution of grades

				(In bales	)			•
Season -	Grade No. 1	Grade No. 2	Grade No. 3	Grade No. 4	Grade No. 5	Grade No. 6	Grade No. 7	Total
1933-34	20,196 15,844 14,655 37,458	1,69,052 1,13,714 1,09,227 1,58,393	90,496 1,29,996 1,43,782 1,56,249	89,642 96,204 1,09,740 1,81,669	1,73,504 1,43,141 1,55,387 1,98,260	2,49,931 2,73,058 3,11,909 3,64,642	7,705 33,126 31,515 30,202	8,00,526 8,05,083 8,76,215 11,26,873 14,71,000 11,16,000

when the full potential supply is forthcoming, would be available for the manufacture of chemical cotton, while the best grades among them may be used in making mattresses, cushions,

pillows, etc.

It is interesting to note, in this connection, that in U.S. A. in the 11 months ending 30 June 1939, 777,000 bales of linters were consumed within the country, while 193,000 bales were exported to foreign countries in the same period. Of the linters consumed within the country, some 400,000 bales were accounted for by those industries which use chemical cotton as their basic material, while the balance must have been consumed in the manufacture of other In the 11 months, preceding June 1940, these figures had increased to 1,070,000 bales for consumption within the States and 305,000 bales for exports to foreign countries, showing the large and profitable trade built up by the U.S. A. from a raw material which until recently has been completely ignored in

We may here consider the point whether India should seek to develop a large export trade for her linters or should endeavour to lay greater emphasis upon the development of chemical cotton industry. We are of the opinion that the latter course is more desirable for the following reasons: Firstly, it will promote the establishment of several industries in which chemical cotton is used, and as we have stated elsewhere these industries form a very large group, whose development will stimulate the consumption of other raw materials. Secondly, if the chemical cotton is prepared in India and exported abroad, the monetary return will be greater than that realized for linters. It is true that the different industries using chemical cotton lay down their special requirements in regard to the grade and quality of the material, and the chemical cotton manufactured for each industry will have to conform to these specifications but this is not a difficult matter and the demands of the consuming industries can be easily satisfied with the help of a small Laboratory.

We will next consider the question as to what extent the Indian linters conform to the American standard grades and to what extent they were defective. The full answers to these questions have been given in this report earlier. may briefly mention here that the quality of linters of the two seasons, 1938-39 and 1939-40, was found to vary considerably, and therefore the same answer cannot be given with regard to linters of these two seasons. The linters of 1938-39 were, on the whole, of a much poorer quality than those of 1939-40, which showed noteworthy improvement both in grade and colour over those of the previous season. The linters of 1938-39 season conform mostly to American grades 4, 5 and 6, while those of the 1939-40 season conform mostly to the American grades 2, 3 and 4. As has been explained in the report, this comparison between the Indian linters and the American grades must be regarded as of a very general nature as there were important differences between the two in regard of colour and the type and quantity of trash present in them. The colour of the American grades ranged from creamy white to brownish red, while that of the Indian linters was mostly greyish white or dirty grey. The differences in the type and quantity of trash present in them were even more remarkable. The trash in the Indian linters, which in some cases was present in considerable quantities, consisted of cut and whole seed, immature seeds or motes, boll rests, leaf bits both large and peppery, and dirt and sand. On the other hand, the American standard grades were conspicuously free from seed and the trash consisted mostly of fine seed-coat bits, small leaf and dirt. As has been pointed out in the report, it is not always an easy matter to remove fine particles of sand and dirt, which adhere to the linters in the washing process, and, therefore, if this

type of trash persists in large quantities in the Indian linters, it is likely to result in high ash content of the chemical cotton which would make it unacceptable to several industries. Similarly, the presence of cut and whole seeds would bring about an appreciable reduction in the yield of chemical cotton from a given quantity of linters, thereby depressing their market value. Consequently, if the Indian linters are presented in the market in a state of reasonable freedom from cut and whole seed and dirt and sand particles, they would conform more nearly to the American standard grades and would be taken up more readily and at higher prices by the consuming industries or the exporters. It is gratifying to note in this connection that the linters of the 1939-40 season already show good improvement in these respects, and we hope that this improvement will be maintained in the future. The differences in colour are comparatively unimportant as they would disappear in the bleaching process to which the chemical cotton is normally subjected.

We will next consider the questions of cost of production of linters per lb. and the price per lb. free on rail at the main centres in India. As regards the cost of production inquiries were made from a number of factories in India which are producing linters but many of them expressed their inability to give this information. Three factories, however, very kindly furnished information on this point. According to two of them the cost of production of linters in India is about one anna per lb. or about Rs. 5 per md. of linters, while according to the third factory the cost of production is 6.44 pies per lb. or Rs. 2.12 per md. These figures show fair variation and it is interesting to note that the linters of which the cost of production is given as Rs. 2-12 per md. were judged, in surface grading, as 'dirty yellow' or 'very dirty yellow' and were found to contain either fair quantity of brown leaf or large quantity of leaf and some crushed seed. Taking these factors into account, we are of the opinion that a cost of production of 11 to 12 pies may be taken as reasonable provided the linters are produced in a fairly clean state.

One of the firms which gave the cost of production as one anna per lb. very kindly supplied the following interesting details. According to this firm the cost of production is made up of (1) power, (2) labour, (3) reduction in weight of the resulting cotton seed, and (4) depreciation and interest on machinery. The cost of treating the seed to one cut is about one anna per maund, while the resulting loss in weight is about  $3\frac{1}{2}$  lb. The value of this

loss in weight is  $10\frac{1}{2}$  pies which, added to the working costs gives one anna a lb. or about Rs. 5 per md. as the cost of production. In the case of certain types of seed no premium is obtained for delinted seeds, but in the case of other types of seeds, e.g. P.A. 4F a premium of  $1\frac{1}{2}$  to 2 annas per md. is obtained for the delinted seed which largely compensates for the cost of treatment in the case of such seed. This cost does not take into account the cost of baling the linters which is about Rs. 2-8 per 5 md. bales or  $1\cdot 2$  pies per lb. of linters.

As regards the price per lb. free on rail at the main centres in India, this would depend, apart from the quality of linters, upon such factors as the demand and available supply at any given time, the distance from the point of production, the price of short staple cotton, the special uses such as the manufacture of gun cotton, explosives, etc. to which, in times of emergency, the linters may be put. The price of linters, therefore, may fluctuate within fairly wide limits owing to these economic factors. This will be seen by the course of prices of American linters between 1930-31 and 1936-37 which are reproduced in Table XVI. The prices of the Indian linters are not available for a number of seasons owing to the infancy of this industry in India. and we are therefore unable to consider their trend in the past few seasons. As regards the samples considered in this report, the prices for the 1938-39 samples were not available in most cases, but we were more fortunate in regard to the 1939-40 samples for which the data were supplied and is given in column 6 of Table XII. It will be noticed that the prices of these samples of linters ranged from Rs. 4-8 to Rs. 8 per md. with the exception of one sample which was priced as low as Rs. 2 per md. the majority of the linters being offered at about Rs. 6 We would naturally expect that the samples which conformed to the higher American grades were offered at relatively higher prices while those which matched the infer or standard grades were offered at lower prices. This is found to be the case in general, but in some cases we came across quite interesting exceptions. For example, sample No. 20 which matched the American standard grade No. 2 was offered at Rs. 4-8 per md. while sample No. 3a which matched the American standard grade No. 4 was offered at Rs. 5-8 per md. apparent anomalies may partly be due to the different quantities of trash associated with these samples, those having a larger amount of trash being naturally valued at a lower rate than the cleaner ones, but they may also be due to some extent at least to the fact that no systematic grading of Indian linters has yet been carried out. The individual sellers, therefore, offer their linters partly on the basis of their cost of production which, as we have seen above, may vary considerably, and partly with an eye to the speedy disposal of their produce. Thus, the price obtained by them may not, in all cases, bear an exact relationship to the quality of the material.

We will next endeavour to compare the prices of the Indian linters with those of the American linters. Such a comparison is neither very easy nor reliable in the present state of our knowledge on account of the following reasons. The grading of American linters has been done on a sound and businesslike basis, and a set of standards is prepared each year which reflects truly the character, grade and colour of the bulk of the linters produced Thus, any sample of the American in U.S.A. linters, which is offered in the market, can be readily matched against these standard grades, and its price can be easily fixed in terms of the basic price of the particular grade which lies nearest to it. The Indian linters, however,

cannot be readily matched against the American standards because of important differences both colour and the type and quantity of Thus, it would present in them. be quite possible that a sample of Indian linters may conform in general appearance to one of the American standard grades, but may contain larger quantities of crushed or whole seed or sand and dirt, which would certainly detract from its value. Furthermore, in fixing the American standards of linters some regard is paid, especially in the superior grades, to their staple length. In the Indian linters, however, this characteristic has not so far received any serious attention. These important differences between the Indian linters and the American standard grades render an exact comparison between them rather difficult, but if these differences are borne in mind, we may make a general comparison. The average prices of the American grades of linters for the eight seasons 1929-30 to 1936-37 are given in Table XVII.

TABLE XVI
Average prices per lb. in cents.

				Grade			
Year	1	2	3	4	5	6	7
1930-31 · · · · 1931-32 · · · · 1932-33 · · · · 1933-34 · · · · · 1935-36 · · · · 1935-36 · · · · · 1936-37 · · · ·	4·29 3·03 2·97 5·45 6·34 6·08 6·32	3.59 $2.52$ $2.52$ $5.10$ $5.71$ $5.49$ $5.80$	2.98 1.93 1.98 4.51 5.18 5.01 5.25	$\begin{array}{c} \cdot \\ 2 \cdot 05 \\ 1 \cdot 31 \\ 1 \cdot 52 \\ 3 \cdot 93 \\ 4 \cdot 65 \\ 4 \cdot 42 \\ 4 \cdot 64 \end{array}$	$     \begin{array}{r}       1 \cdot 63 \\       1 \cdot 04 \\       1 \cdot 24 \\       3 \cdot 57 \\       4 \cdot 28 \\       3 \cdot 94 \\       4 \cdot 18 \\    \end{array} $	$ \begin{array}{r} 1 \cdot 24 \\ 0 \cdot 83 \\ 1 \cdot 04 \\ 3 \cdot 25 \\ 4 \cdot 00 \\ 3 \cdot 43 \\ 3 \cdot 79 \end{array} $	1·01 0·66 0·85 3·06 3·76 2·92 3·29

TABLE XVII

Price of American linters

Grade No.	Price in rupees per md.	Price in dollars per md.
,	Rs. as. ps.	4.16
$\frac{1}{2}$	12 4 10	3.69
3	10 9 3	3.18
4	8 13 8	$2 \cdot 65$
5	7 13 6	2.36
6	6 12 6	2.03
7	5 14 11	1.78

Referring to Table III we observe that majority of the samples of Indian linters conform, at least in general character, to the American

grades 2 and 3, except that the Indian linters contained larger quantities of trash in the form of leaf, crushed and whole seed, boll rests, etc. as will be seen from the last column in Table XII in which the nature and amount of these impurities are described briefly. On referring to Table XVII again, we notice that the average prices of the American standard grades Nos. 2 and 3 were Rs. 12-4-10 and Rs. 10-9-3 per md. respectively. If we compare these with the prices of the Indian linters, namely Rs. 6 to Rs. 7 per md., we see that the latter have beer. offered in the market at much lower prices that those obtaining for the American linters of approximately corresponding grades. Part of this difference is no doubt due to the larger quantities of trash present in the Indian linters, but even after making due allowance for this factor, we cannot help feeling that owing to lack of proper grading, inadequate demand in the country

and the absence of suitable marketing facilities, the Indian linters have been sold at lower prices than they should have fetched according to their grade and quality. We are of the opinion that if the delinting of Indian seeds is improved, as can be done readily, so as to reduce the quantity of trash in the linters, and if a proper set of standard grades is prepared each year and the linters are graded as is done in America, the producers would get much better prices for their raw material.

We will finally consider briefly the machinery which is used in the production of linters. The seed cotton is, of course, ginned either on a roller gin or a saw gin and the seed thus obtained, which contains from 1.5 to 4 per cent of linters, is then passed through a delinting machine. From the information which has kindly been supplied by the factories, it appears that three types of delinting machines are being used at present in India. These are the Continental, Carvers and Verner machines. In addition, one of the factories used a country made delinting machine, and it is interesting to note that the quality of the linters produced on this machine was not very inferior to that of the linters produced with the help of the imported machines. In most cases the Indian factories produced the quality of linters known as first cut and therefore their yields per ton of seed were rather small as compared with the American yields. It is, however, highly probable that the machines used by them can be employed with suitable changes in settings, etc. for producing either mill run or second cut linters as well, though there appears to be some prejudice, as stated by one of the factories, against treating the seed to a second cut on the grounds that such linters have a bad colour and that their production involves greater wear and tear of the machinery.

VI. CONCLUSIONS

An attempt has been made to grade 39 samples of Indian linters belonging to the 1938-39 and 1939-40 seasons by three different methods, with a view to evolving a quick and reliable method of grading Indian linters. These methods were (a) mechanical analysis, (b) chemical analysis and

(c) surface grading.

The Indian linters of the 1938-39 season showed a fair amount of variation in their quality, some of them containing appreciable quantities of trash in the form of leaf, stalk or seed, the last of them being whole, crushed or undeveloped. The linters of the 1939-40 season show distinct improvement over those of the preceding year in respect of grade and quality, and this feature constitutes a hopeful sign for the future of the chemical cotton industry in India.

A comparison of the results of the mechanical and chemical treatments has shown the existence of a relationship between the fibre content and the percentage of chemical cotton in a sample. This relationship improved by substituting dry alphacellulose for the chemical cotton. A further improvement resulted from substituting total fibre content of the sample, i.e. the recovered fibres plus the short fibres or fuzz lost in the form of invisible loss in the formula. The relationship, as finally obtained, between the total fibre content and the dry alpha-cellulose of a sample is as follows:

$$F + f - K = \alpha$$

Where F is the fibre separated by the Shirley Analyser, f is the percentage of fibres below  $\frac{1}{16}$  in given by the invisible loss, K is a constant being equal to  $16 \cdot 9$  and  $\alpha$ , the dry alpha-cellulose content of the air dry linters.

The average difference between the values of alpha-cellulose content actually obtained by experiments and those calculated with the help of the formula was found to be only  $2 \cdot 3$  per cent.

The validity of the formula was also tested by determining, on the one hand, the dry alphacellulose content of American Standard Grades of linters, and, on the other, by calculating it with the help of the formula. A close agreement was observed between the two sets of values.

It was found by actual determination of the mean length of fibres in different group lengths in the standard linters that the  $\frac{1}{16}$  in. group length represents very closely the invisible loss in the Shirley Analyser. Thus, the mechanical analysis gives an indication of the staple of the sample, as the percentage of fibres having length less than  $\frac{1}{16}$  in. goes on diminishing steadily as the staple of the sample increases from grade 7 to grade 1.

A new method based on the mechanical analysis is suggested for grading linters which is capable of giving reliable information as regards the alphacellulose content and the staple of a given sample.

The results of the surface grading method have been compared with those obtained by the proposed method. The causes for the observed differences are discussed, and it is pointed out that these serve to emphasize the desirability, even the necessity, of adopting the proposed method for grading linters where testing facilities are available, in preference to the surface grading method.

The economic aspects of the production of linters of different grades in India have been considered. It is estimated that a potential supply of some 80,000 bales of linters of 400 lb.

each already exists in the country, but in order to get at a more correct figure, the need for a properly conducted survey is stressed. It is surmised that a large proportion of this supply would be available for the manufacture of chemical cotton, while the best grades among the Indian linters may be used for stuffing mattresses, cushions, pillows, etc. It is recommended that every effort should be made to develop the chemical cotton industry and the allied industries within the country rather than to export the raw linters to foreign countries.

The cost of production of linters in India in normal times has been investigated and is estimated to be about one anna per lb., though it must vary with such factors as (a) cost of power, (b) labour charges, (c) reduction in weight of the resulting cotton seed, etc. The price actully realized by the linters bore only a general relationship to their quality, as, in some cases, it was affected considerably by (a) their cost of production and (b) the desire for a speedy disposal. On comparing these prices with those usually

offered for American linters of the corresponding grades, it is concluded that the Indian linters have generally been sold at rather low prices. It is suggested that if the trash percentage in the Indian linters could be reduced by improving the delinting methods, Indian linters would fetch higher prices than they did in the 1938-39 and 1939-40 seasons.

#### ACKNOWLEDGEMENTS

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## APRICOT SEED CAKE AS A NITROGENOUS MANURE

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(With three text-figures)

WILD apricot (Prunus armeniaca Linn., vernacular Zardalu) trees grow abundantly on an extensive area in the Simla Hills. Their fruits are consumed. The seeds contain oil, which is used in cooking, burning, toilet creams, and certain pharmaceutical preparations. The oil is obtained by pressing the kernels from the pits, which are separated from the fruits of Prunus armeniaca in large quantities in the manufacture of dried apricots. The kernels constitute about 20 per cent of the pits and contain from 40 to 45 per cent of oil. The cake left after extracting the seeds for oil is neither utilized as a cattlefood owing to its bitter taste, nor does it find an alternative application as a manure. It is burnt as a fuel only. The chemical examination of the cake by the method of Warth and Ko Ko Gyi [1918] revealed the presence of 0.057 per cent of hydrocyanic acid, which is high enough to prove fatal to the cattle, when fed. The content of hydrocyanic acid may be attributed to the presence of some cyanogenetic compound like glucoside in the cake. It is well known that glucosides are widely distributed in the plant kingdom and the first products of their hydrolysis in the soil are glucose, aromatic compounds, and hydroevanic acid.

The cake however contains a good amount of nitrogen (6·7 per cent), besides a fair proportion of phosphate  $P_2O_5$  (1·49 per cent) and potash  $K_2O$  (1·09 per cent). Its waste as a fuel is therefore an economic loss to agriculture, especially in its relation to the supply of nitrogen for proper crop production.

With a view to find out its possible utilization as a suitable nitrogenous manure, the enquiry has resolved itself into two distinct divisions, viz.

- (1) the biochemical study of its nitrogen transformation in the soil, and
- (2) the determination of its efficiency as a nitrogenous manure.

#### I. BIOCHEMICAL STUDY OF NITROGEN TRANSFOR-MATION IN THE SOIL OF APRICOT CAKE

It is essential to know at the first instance how much of the nitrogen contained in the apricot seed cake can be converted into available forms, before evaluating its efficiency for crop growth in a soil. In order to do this, the cake was subjected to a study of its nitrogen transformation in the laboratory from the biochemical point of view in three different types of soil, viz. (1) a highly calcareous Pusa soil belonging to the trans-Gangetic alluvium, (2) a non-calcareous

Kalianpur soil near Cawnpore, United Provinces, and (3) a hill soil from Solon near Simla Hills. The mechanical and the partial chemical composition of these soils are given in Table I in order to give some idea of their water relationship, aeration, and mineralization of organic matter, etc.

Table I

Mechanical and partial chemical composition of

Pusa, Kalianpur and Solon soils

Constituents per cent	Pusa soil	Solon soil	
I. Meche	anical compo	sition	
Clay	5·62 23·68 47·37 23·33	$\begin{vmatrix} 12 \cdot 14 \\ 24 \cdot 80 \\ 28 \cdot 44 \\ 34 \cdot 62 \end{vmatrix}$	$ \begin{array}{ c c c } 19.96 \\ 34.48 \\ 18.98 \\ 26.58 \end{array} $
II.	Chemical con	iposition	

				1
Organic matter .		2.05	0.72	4.34
Carbon · ·	•	0.46	0.42	$2 \cdot 52$
Nitrogen		0.315	0.072	0.17
Carbon/Nitrogen ratio	٠.	14.06	5.80	14.80
Calcium carbonate .	•	35.00	0.50	6.40
pH		8 · 29	7.63	8.19
Water-holding capacity		48.00	$52 \cdot 64$	50.10
			l	

Forty milligrammes of nitrogen in the form of cake were applied per 100 gm. of air-dry soil and

16 per cent of moisture was maintained throughout the experiments. The incubation carried out at the ordinary temperature which varied between 29° and 33°C. in the case of Pusa and Kalianpur soils, and between 21° and 31°C. in the case of Solon soils. Periodically samples of soil were drawn and ammonia, nitrite, and nitrate determined in them,-ammonia by the aeration method of Matthews [1920], nitrite and nitrate by the colorimetric methods with sulphanilic acid and α-napthylamine, and with phenoldisulphonic acid respectively. Controls were run simultaneously side by side in the same soils without any application of the cake. While calculating the availability of the cake with respect to different forms of nitrogen as given in the following tables, due allowance was made by subtracting the corresponding values of available nitrogen formed in the controls under identical experimental conditions. The results for Pusa, Kalianpur, and Solon soils are set forth in Tables II and III.

From the data presented in Tables II and III it is evident that 63 and 60 per cent of nitrogen contained in the apricot seed cake have been transformed into available forms by 8 weeks' incubation in Pusa and Kalianpur soils respectively, and 54 and 58 per cent nitrogen of the original and the fat-free cake respectively in Solon soil by about 7 weeks' incubation. These amounts represent the maximum limit of transformation under the experimental conditions as further incubation does not seem to increase'

Table II

Nitrogen changes of apricot seed cake in Pusa and Kalianpur soils

Num-		Cake in Pu	ısa soil			Cake in Kali	anpur soil	
ber of days incu- bated	Mg. N as NH <sub>3</sub>	Mg. N as NO <sub>2</sub>	Mg. N as NO 3	Totals	Mg. N as NH <sub>3</sub>	Mg. N as NO <sub>2</sub>	Mg. N as NO <sub>3</sub>	Totals
Nil 7 10 144 17 211 255 31 355 422 499 566 70 81 100	0.40 $15.68$ $17.76$ $20.24$ $21.36$ $21.44$ $20.24$ $15.76$ $13.04$ $10.00$ $7.52$ $6.24$ $5.04$ $4.80$ $4.64$	Nil0.030.01 0.02 0.05 0.26 0.74 1.57 0.82 0.01 Trace "" ""	$\begin{array}{c} -0.36 \\ -0.36 \\ -0.36 \\ -2.16 \\ -0.72 \\ -1.08 \\ -0.78 \\ -1.44 \\ 2.98 \\ 8.28 \\ 11.88 \\ 14.40 \\ 18.72 \\ 18.82 \\ 18.72 \\ 19.26 \end{array}$	0·04 15·29 15·59 19·54 20·33 20·92 19·54 20·31 22·14 21·89 21·92 24·96 23·86 23·52 23·90	0.32 $6.96$ $3.04$ $1.04$ $0.72$ $0.32$ $0.24$ $0.32$ $0.16$ $0.32$ $0.16$ $0.32$ $0.08$	Nil 1·39 1·05 0·07 0·04 0·01 Trace	$\begin{array}{c} -0\cdot 27 \\ -1\cdot 80 \\ 4\cdot 66 \\ 16\cdot 56 \\ 16\cdot 74 \\ 17\cdot 82 \\ 20\cdot 52 \\ 20\cdot 88 \\ 22\cdot 14 \\ 23\cdot 40 \\ 23\cdot 76 \\ 23\cdot 40 \\ 23\cdot 40$	0·05 6·55 8·75 17·67 17·50 18·15 20·84 21·12 21·20 22·46 23·56 24·08 23·48 23·28

Table III

Nitrogen changes of apricot seed cake in Solon soil

Num- ber of	Ori	ginal cake in S	Solon soil	1		Fat-free	coake in Solor	n soil
days incu- bated	Mg. N as NH 3	Mg. N as NO <sub>2</sub>	Mg. N as NO 3	Totals	$^{ m Mg.~N}_{ m as~NH}_{ m 3}$	$rac{ m Mg.~N}{ m as~NO_2}$	Mg. N as NO 3	Totals
Nil 3 - 5 7 10 14 21 28 45 55 70 84	0·08 5·28 9·12 7·04 0·64 0·24 Nil	-0·01 0·09 0·32 0·65 0·03 Nil " " " "	-0·18 -1·26 -0·36 3·96 15·52 16·66 19·08 19·35 21·60 21·24 21·24 21·42 20·98	-0·11 4·11 9·08 11·65 16·19 16·90 19·08 19·35 21·60 21·24 21·24 21·42 20·98	Nil 9·04 12·40 8·72 0·96 0·24 Nil ""	-0·01 0·12 0·39 0·70 0·02 Nil	$\begin{array}{c} -0.18 \\ -1.17 \\ 0.50 \\ 5.76 \\ 20.38 \\ 20.62 \\ 21.87 \\ 23.04 \\ 23.40 \\ 23.40 \\ 23.58 \end{array}$	

the available nitrogen in the soil mixtures. In the fat-free mustard cake Walton [1928] found that about 60 per cent of its nitrogen nitrified in Pusa and Kalianpur soils in 8 weeks' incubation, which substantially agrees with the results obtained in the nitrification of apricot seed cake and points to its suitability as a nitrogenous manure like mustard cake. In the controls, the amount of soil nitrogen nitrified in Solon soil is the least, being about 4 mg. only, and in Kalianpur and Solon soils the amounts nitrified are about 9 and 14 mg. respectively.

A closer examination of the data reveals some interesting points with regard to the manner in which the nitrogen changes takes place in these soils.

#### PUSA SOIL

Nitrogen changes of the cake in Pusa soil are shown in Fig. 1.

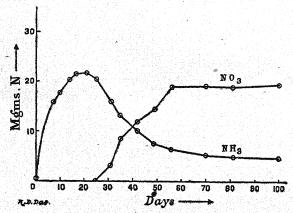


Fig. 1. Nitrogen changes of apricot seed cake in Pusa soil

There is an abundant formation of ammonia from the cake in Pusa soil from the very start, which sharply increases and reaches the maximum in the first three weeks' incubation. Then it rapidly falls off during the next five weeks, after which it slackens and then remains practically constant till the 14th week's incubation, when the evolution of about 5 mg. of nitrogen as ammonia still persists. The immediate effect of the production of hydrocyanic acid as a result of hydrolysis of the cake and of ammonia in the cake-treated soil appears to have completely retarded the formation of nitrate for the first  $3\frac{1}{2}$  weeks and also of nitrite for about two weeks. The negative values in Table II point to the fact that in the cake-treated soil there is a lesser production of nitrite and nitrate than in the control (soil alone) for the periods of time noted therein due perhaps to the toxic effect of hydrocyanic acid produced on the nitrifying flora of the soil. After  $\overline{3}\frac{1}{2}$  weeks the nitrate formation rapidly pushes forward reaching the maximum in 8 weeks accompanied by a gradual fall of ammonia, and then remains practically constant till the 14th week's incubation. After the second week the amount of nitrite formed rapidly increases and reaches the maximum in  $4\frac{1}{2}$  week's incubation. Then it slopes down quickly to a negligible quantity after the 5th week.

#### KALIANPUR SOIL

Nitrogen changes of the cake in Kalianpur soil are shown in Fig. 2.

In the cake-treated Kalianpur soil the accumulation of ammonia is considerably less in the initial stages of incubation than in the cake-

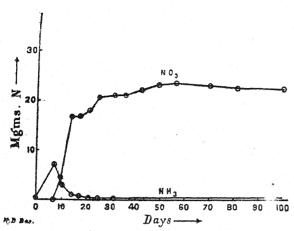


Fig. 2. Nitrogen changes of apricot seed cake in Kalianpur soil

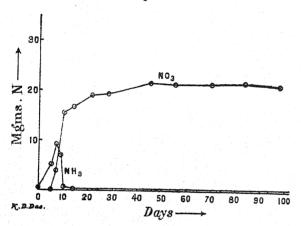


Fig. 3. Nitrogen changes of apricot seed cake in Solon soil treated Pusa soil. Its retarding effect, if any, coupled with the production of hydrocyanic acid as a result of hydrolysis of the cake, on the formation of nitrate is pronounced in the 1st week's incubation. In the next week there is a sharp fall in the recumulation of ammonia and the curve of its formation solpes remaining practically constant after the 3rd week. The nitrate formation curve, on the other hand, initially lagging behind the control during the 1st week as shown by the negative values of nitrate in Table II for that period, shoots up steeply for the next 21 weeks. Then it slackens reaching the maximum in the 7th week and later on remains practically constant till the 14th week's incubation.

#### SOLON SOIL

Nitrogen changes of the cake in Solon soil are shown in Fig. 3.

In the cake-treated Solon soil the cake simulates its behaviour in Kalianpur soil. Here an

experiment with a fat-free sample of the cake was included in order to determine its superior action, if any, to the untreated cake during the course of nitrogen transformation. The original cake contained as much as 10 per cent of oil, which is believed to retard the action of soil organisms on it. Although the amount of nitrogen converted into available forms is only 4 per cent higher in the fat-free sample than in the original cake, the course of action is similar in both the cases and follows the same relationship between ammonia and nitrate formation as in the case of Kalianpur soil.

A critical examination of the nitrogen changes reveals an initial production of hydrocyanic acid from the cake, the immediate depressing effect of which is apparent on the formation of both nitrite and nitrate in all the three soils experimented with. This effect however dissipates in about a week's time in Kalianpur and Solon soils, and in 3½ weeks in the calcareous Pusa soil.

There is some controversy on the depressing effect of ammonia on nitrifying organisms. Boulanger and Massol [1905] found that the growth and activity of nitrate bacteria is not injured by the ammonium salt, but by free ammonia. Omeliansky [1900] and Meyerhoff [1917] observed that a high concentration of ammonia or nitrite is harmful to the progress of bacterial nitrification. On the other hand, later workers, such as, Fred and Davenport [1921], and Joshi [1928] found that nitrifying organisms can tolerate much larger amounts of organic matter and ammonical nitrogen than those given by the early investigators. Hydrocyanic acid thus appears to be mainly responsible in exercising a toxic effect on the normal activities of nitrite and nitrate bacteria in the soil in the initial stages of incubation, but, later on, this injurious effect is minimized and ultimately disappears owing to the dissipation of hydrocyanic acid. Then the conversion of ammonia into nitrate follows by the gradual preponderance of nitrifying organisms.

## II. EFFICIENCY OF APRICOT SEED CAKE AS NITROGENOUS MANURE

It has already been demonstrated above that 54 to 63 per cent of the nitrogen present in the cake is transformed into available forms in three different types of soil as shown in Tables II and III in about two months. It therefore became of interest to determine how far this available nitrogen derived from the cake could be utilized for growing crops in these soils, and, as a consequence, if this cake could find an application as a suitable nitrogenous manure for soils, especially in the neighbourhood of its production.

In order to test this, a series of pot experiments was started in the winter of 1932 with the calcarous Pusa soil. Wheat was chosen as the rabi (winter) crop which responds readily to nitrogenous manures. Four pots of similar dimensions, e.g. 9 in in diameter by 12 in high formed a group and received similar treatment. The pots contained 15 kilos of air-dry soil in each and 16 per cent of moisture was maintained in the soil throughout the experiment. The apricot seed cake was applied to the pots according to the following scheme, given in Table IV.

Table IV

Scheme of application of apricot seed cake to pot-culture experiments

<b>.</b>	W.		anurial const	
Pot numbers	Mg. nitrogen per kilo of soil	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
1 to 4 5 to 8 9 to 12 13 to 16 17 to 20	Control 10 20 40 50	20 40 80 100	4·47 8·94 17·88 22·35	3·27 6·54 13·08 16·35

Pusa Wheat No. 12 was sown on 10 November 1932 and the crop harvested on 20 March 1933. In order to determine how far the residual effect of the manure persisted in Pusa soil, a second crop of ragi (Eleusine coracana) was raised in the following summer in these very pots without any further manurial treatment. Ragi seeds were sown on 11 June 1933 and the crop was harvested on 3 October 1933.

Although the weights of both grain and straw per pot were recorded separately, only the mean yields of grain per treatment for both wheat and ragi are given in Table V along with the standard errors calculated by Fisher's [1932] analysis of variance. As a matter of fact, similar conclusions emerge from the examination of data either for grain or straw.

In order to obtain further confirmatory data, pot experiments were instituted in the winter of 1933 with Solon and Kalianpur soils. Similar pots were used and the same treatments followed as in Pusa soil. Pusa Wheat No. 12 was sown on 3 November 1933 and the crop raised harvested on 14 March 1934. The mean yields of grain per treatment both for Solon and Kalianpur soils are given in Table VI along with the standard errors calculated by Fisher's [1932] analysis of variance.

TABLE V

Effect of apricot seed cake as a nitrogenous manure in Pusa soil in 1932-33

	Prima	ry effect	Residu	al effect	
Mg. N per	Wheat 19	932-33	Ragi 1933		
kilo of soil	Mean yield in gm.	Per cent increase over control	Mean yield in gm.	Per cent increase over control	
Control 10 20 40 50	$4 \cdot 34$ $8 \cdot 01$ $8 \cdot 26$ $10 \cdot 08$ $11 \cdot 06$	84.6 90.4 132.3 155.0	9.08 10.63 10.13 10.55 12.20	17·1 11·6 16·2 34·4	
Standard er compariso yields	ror for on of mean	0.59	0.	98	
Critical di for $P=1$ for $P=5$	per cent	1·74 1·26		89 09	

TABLE VI

Efficiency of apricot seed cake as a nitrogenous manure on the yield of wheat in Solon and Kalianpur soils in 1933-34

	Solon	soil	Kalianp	ur soil
Mg. N per kilo of soil	Mean yield in gm.	Per cent increase over control	Mean yield in gm.	Per cent increase over control
	15.46		0.00	
Control	12.43	10.9	6.08	94.1
$\frac{10}{20}$	$14.83 \\ 15.88$	19·3 27·8	17.03	180-1
40	21.80	75.4	19.63	222 8
50	$\begin{array}{c} 21.00 \\ 24.08 \end{array}$	$93 \cdot 7$	24.18	<b>3</b> 00 · 0
Standard e comparis yields	rror for on of mean	1 · 49		1.79
Critical o	lifference			
for $P=1$	per cent	4.39		5 · 28
	per cent	3.18		3.82

From a consideration of the cropping results of the three soils, it is evident that the increase in crop yield over the control is high with every treatment of the cake. In the case of Pusa soil two

crops were raised after a single application of the cake, viz. (1) wheat in the winter of 1932 followed by (2) ragi in the following summer of 1933, the latter crop showing the residual effect of the manure. Cropping results showed that the increase in the yield of wheat over the control was considerable according to the increasing doses of the manure applied and that the differences in mean yields of grain between control and every other treatment were significant being greater than the critical-value of difference even for one per cent level of significance. The maximum crop was obtained by the application of 40 mg. of nitrogen per kilo of soil as apricot seed cake, although a little better crop was produced by 50 mg. of nitrogen per kilo which was not however statistically significant.

In the case of ragi crop, although the increase in the yield of grain over the control indicated the residual effect of every treatment of the cake, the residual effect of 50 mg. of nitrogen only gave significantly higher yield than the control.

It may therefore be concluded that the residual effect of the cake can persist till the next succeeding crop in calcareous soils, only when it is initially applied at the rate of 50 mg. of nitrogen per kilo of soil.

In the case of Solon and Kalianpur soils the increase in the yield of wheat over the control was high with the increasing doses of nitrogen applied in the form of apricot cake. The differences in mean yields of grain between control and every other treatment of the cake were also highly significant as in the case of Pusa soil except for 10 mg. of nitrogen in Solon soil showing no significant increase. It will be further noticed that the application of 40 and 50 mg. of nitrogen per kilo of soil gives the maximum yield of wheat in Solon and Kalianpur soils respectively.

A closer examination of the cropping results of the three soils reveals the fact that the maximum crop response was attained to the application of apricot seed cake in Kalianpur soil, next came in order the Pusa soil, and last of all was the Solon soil. It may therefore be concluded that apricot seed cake can serve as a suitable nitrogenous manure perhaps for various types of soil, and especially in agricultural lands in the neighbourhood of Solon where it is available in plenty, but at present wasted as a fuel only.

#### SUMMARY AND GENERAL CONCLUSIONS

1. About 60 per cent of nitrogen present in the apricot seed cake may be transformed into available forms in Kalianpur, Pusa, and Solon soils on 7 to 8 weeks' incubation.

2. Of the soil nitrogen present, the least amount is nitrified in Solon soil which nevertheless con-

tains three times as much organic nitrogen as in the other soils; the highest nitrification takes place in Pusa soil, and Kalianpur soil stands midway in this respect, the soil nitrogen nitrified being 4, 14, and 9 mg. respectively. The same order of the nitrification of the cake too is maintained in these soils.

- 3. In all the three soils a large evolution of ammonia takes place initially from the cake. Later on, however, it gradually goes down to a small quantity.
- 4. An appreciable quality of hydrocyanic acid is found in the cake, indicating the presence of some cyanogenetic compound in it. Its content is high enough to prove fatal to the cattle, if the cake is fed to them.
- 5. Hydrocyanic acid is a product of hydrolysis of the cake in the soil. It might have contributed towards retarding the formation of both nitrite and nitrate in the early stages of incubation. This injurious effect is however later on reduced to a minimum and ultimately non-existent.
- 6. Pot experiments showed that the application of 40 mg. of nitrogen as cake per kilo of soil produced the maximum yield of wheat in Pusa and Solon soils, and 50 mg. in the case of Kalianpur soil. The results were found to be statistically significant.
- 7. In Pusa soil the increased yield of wheat over the control was high according to the increasing doses of the cake varying from 10 to 50 mg. of nitrogen per kilo of soil. The residual effect of 50 mg. of nitrogen applied initially per kilo of soil gave significantly high yield of rapi (Eleusine coracana) over the control, the rest of the treatments giving somewhat higher yields than the control, though not statistically significant. The residual effect of the cake was not however tested in Solon and Kalianpur soils.
- 8. In Kalianpur soil the increased yields of wheat over the control were remarkably high with varying doses of the cake.
- 9. In Solon soil the yields were the least, compared to Pusa and Kalianpur soils. The results were however statistically significant except for 10 mg. of nitrogen per kilo of soil.
- 10. The cake being thus effective as a nitro genous manure with an important crop like wheat in three dissimilar types of soil, it will undoubtedly prove as an efficient nitrogenous manure for soil which are particularly deficient in this constituent, and especially so in agricultural lands in the neighbourhood of Solon where it is available in plenty, but at present wasted as a fuel only,

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## STUDIES ON THE ROOT-ROT DISEASE OF COTTON IN THE PUNJAB

XIV. EFFECT OF SOIL TREATMENT ON DISEASE INCIDENCE

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(With one text-figure)

Root-rot of cotton is the most destructive disease of this crop in the canal irrigated colonies of the Punjab. The soils in these areas are predominantly light loam in texture with 15-20 per cent clay, and are mostly alkaline in reaction. The degree of alkalinity varies considerably and may even go up to pH 9 or so in some highly alkaline soils. The causal organisms Rhizoctonia Solani Kuhn and Macrophomina phaseoli (Maubl.) Ashby [Rhizoctonia bataticola (Taub.) Butler] are soilinhabiting fungi and bring about the death of the plants by attacking their roots. It was considered possible therefore to control the disease by alteration of soil conditions as affecting the host and the organisms. The experiments reported in this paper were conducted with this end in view during 1935-1940. Such methods of control are directed to the eradication of the fungus from the soil during its resting period, and the control of the disease may be effected by checking the activity of the fungus during its parasitic phase, by increasing resistance of the host, and by creating adverse soil conditions for the sub-soil development and parasitism of the organism concerned.

Such methods have proved successful in the control of several plant diseases. The most familiar example is that of the control of potato scab by application to the soil of chemicals or fertilizers with a view to increasing soil acidity. The severity of root rot of wheat seems to be influenced by the time and mode of tillage [Sewell and Melchers, 1924], and the use of fertilizers has been shown to result in the control of root-rot of peas [Haenseler, 1930]. Neal [1928], Miles [1929,

1930] and Young and his associates [1932] have suggested control of *Fusarium* wilt of cotton by addition of potassium salts to the soil.

#### EXPERIMENTAL

All the experiments reported herein were conducted at Lyallpur on land which was heavily and uniformly infected with the disease in order to get reliable and comparable data. The distribution of infection in a large number of fields had been mapped out during the previous seasons. Fig. 1 shows the method of mapping. Black lines in the figure indicate the positions of plants killed by root-rot. Cotton was sown in the month of May which is the most favourable period for development of the disease. Both Desi (Gossypium indicum) and American (G. hirsutum) cottons were used in these experiments, and sowings were done in lines 2½ and 3 ft. apart respectively

A. Effect of soil fumigation

In 1937 an infected field was divided into 18 plots each 12 ft. × 12 ft., and holes I ft. deep were bored with a soil sampler at a distance of 2 ft. from one another. Altogether 36 holes were bored in each plot. Paradichlorobenzene or calcium eyanide was added in the holes at the rate of 340 lb. per acre about two months before planting cotton. In the control plots the holes were bored and filled up with soil without the addition of any chemical. Six plots were treated with each of the chemicals and six were kept as controls. The experiment was laid out on a randomized block system. Desi cotton variety Mollisoni 15 was sown on 15 May 1937, and observations on the incidence of the disease were made at weekly intervals throughout the growing season. The data are summarized in Table I,

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#### SQUARE 48 PLOTES

SHOWING THE ACTUAL POSITION OF GOTTON PLANTS HILLES ET ROOT ROY

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Fig 1
TABLE I
Effect of soil fumigation

Treatment	Total No. of plants	Total plants killed	Average per cent mortality	Average yield of seed cotton per acre
Calcium cyanide	218 191 226	67 10 69	28·32 4·67 29·53	lb. oz. 425 2 374 15 621 2

It was observed that the disease incidence in the plots treated with paradichlorobenzene was appreciably reduced, but germination in all these plots was considerably delayed and the plants remained comparatively much smaller and stunted. It is therefore difficult to explain as to whether low incidence of the disease in the plots treated with paradichlorobenzene was due to this treatment or to late germination which amounts to late plantings in the month of June. It has already been shown [Vasudeva, 1937; 1943] that the disease can be controlled by planting cottons late towards the end of June. Yield of seed cotton in the treated plots was lower than that obtained from the controls.

In laboratory tests made with calcium cyanide [Vasudeva, 1937] it was observed that hydrocyanic acid gas penetrates up to a depth of 18 in. after six days and kills the resting bodies of the fungi concerned. It appears from the

results recorded in Table I that this chemical, when tested under field conditions, almost failed to check infection, and this failure was probably due to the inability of the disinfectant to reach the root rot organisms in the deeper layers of the soil. Fumigation of the soil by means of chemicals appears to be unsatisfactory because of the cost involved and the possibility of the disinfectants proving inaffective when applied under field conditions.

B. Effect of cultural treatment

Experiments in connection with the modes of perennation of the fungi causing root-rot showed that the diseased roots of cotton found in affected patches in the field invariably yielded the causal fungi in culture. These organisms were also isolated from roots of cotton plants collected from fields allowed to fallow after removal of the crop, and the resting bodies of the fungi obtained from such roots collected from various localities were

found to be viable when cultured on an artificial medium.

In the summer of 1935 roots of cotton plants bearing the resting bodies of the fungi were left in the open exposed to atmospheric conditions and were also buried in the soil at various depths ranging from one to five feet. These bodies were found to be viable even after three years and isolations from the roots yielded *Macrophomina phaseoli* in all cases, but *R. Solani* was very rarely recovered. The fungi are also capable of over-wintering under frosty conditions. In view of this normal cultural treatments were so varied so as to minimize the source of infection in the soil.

(a) Effect of removal of diseased debris, addition of farmyard manure and flooding.—An experiment involving these treatments singly and in combination with a non-treated variant, i.e., eight variants in all was laid out in 1939-40 on a randomized block system. Five repeats of each treatment were kept.

A heavily infected field was divided into plots, each plot being 24 ft. × 14 ft. Removal of diseased debris was as far as possible effected with the help of plant pullers and then by deep ploughing with a furrow-turning plough and picking up the roots by hand. Altogether, flooding of the plots was done five times before planting cotton. The time required for flooding each line of four plots, during the first three irrigations was 7 to 8 min., and in the last two irrigations only 6 to 7 min. were taken in flooding each line. The time required for giving a normal irrigation, i.e. 21 in, water after sowing cotton was about 4 to 5 min. Well rotted farmyard manure at the rate of 18 tons per acre was added. All precautions were taken to obtain reliable and comparable data.

On 20 May 1939, Desi cotton variety Mollisoni 39 was sown in all the 40 plots. Observations for disease incidence were taken for the first time on 19 June 1939 and thereafter at weekly intervals. The average per cent mortality due to root-rot in various treatments is summarized in Table II

Table II

Effect of different treatments on disease incidence

	Untreated—not flooded			Treated—flooded			
${f Treatment}$	Total No. of plants	Total killed due to root-rot	Average per cent mortality	Total No. of plants	Total killed due to root-rot	Average per cent mortality	
1. Addition of farmyard manure	739	495	69 • 1	747	390	52.0	
2. Removal of diseased debris .	750	494	65.9	748	183	24-4	
3. Removal of diseased debris and addition of farmyard	746	245	32.9	747	404	54.0	
manure 4. Untreated control	736	296	40.1	748	439	58.6	

Indications of reduction in mortality were obtained only in the plots from which cotton roots had been removed and the plots had been flooded. The results, however, were barely significant.

A similar experiment was laid out in 1940-41 and three repeats of each treatment were kept. No indication of any appreciable reduction in mortality in any of the treatments was obtained.

The effect of flooding alone was also studied in an experiment where cotton variety *Mollisoni* 15 was sown in the middle of May on ridges as well as on flat. In controls only normal irrigation was given. No appreciable difference in mortality in the treated and untreated plots was observed.

The effect of removal of old roots on the development of the disease was studied in another experiment in which soil was dug up to a depth of 2½ ft. and from it all pieces of cotton roots were carefully removed. The disease incidence in the plots so treated was 40.7 per cent as compared to 80 per cent in the untreated control.

(b) Effect of trenching. The experiment was conducted in plots, 30 ft.×17 ft. each. About six weeks before sowing cotton the soil in two plots was dug up to a depth of 6 ft. and in another two plots digging was done only to a depth of 4 ft. After a couple of days the earth was turned over and replaced in the pits so dug. All the plots were then irrigated. No digging was done in the control plots. American cotton variety 4F was sown on 23 May 1938, and observations on the incidence of the disease were made weekly. The data are set out in Table III.

TABLE III Effect of trenching on disease incidence

Treatment	Total plants	Total killed	Average per cent mortality	Average out turn of seed cotton per acre
1. Trenched 6 ft	386 325 358	35 142 176	9·0 42·4 48·1	lb. oz. 1262 12 706 5 624 3

The data clearly show that the incidence of root-rot was greatly reduced by trenching up to a depth of 6 ft. and that the outturn of seed cotton in the trenched plots was considerably increased. The general growth of plants in the trenched plots was very vigorous. This experiment was repeated for two consecutive years with similar results but, as this method of control is very laborious, it is not likely to be adopted in

(c) Effect of tillage.—An experiment to test the effect of tillage on the incidence of rootrot disease was laid out at the British Cotton Growers Association's Farm at Khanewal. Three isolated fields, each 190 ft.  $\times$  110 ft. in which cotton plants had died of root-rot in the previous seasons, were selected. field was divided into five plots, and each plot was 110 ft. × 38 ft. Five treatments, i.e., continuous cropping with cotton, summer tillage, winter tillage, 12 months tillage, and 24 months tillage were replicated three times. Before sowing all fields were cultivated by a furrow-turning plough at weekly intervals throughout the period of the experiment. Every effort was made to plough to a depth of 6 in and to remove all weeds. Occasionally rain hampered these operations, but the average of one ploughing per week was always made up.

In the plots where cotton was continuously cropped, only normal cultivation was given, and the test crop of Desi cotton var. Mollisoni 15 was planted in May 1935, 1936 and 1937, according to local cultural practices. The soil in the summer and winter tillage plots was ploughed weekly during April to September, 1935, and October to February, 1935, respectively. The cultivation in the 12 months tillage plots was carried out from April 1935 to April 1936. Test crop in all such treated plots was sown in May 1936. In the 24 months tillage plots weekly cultivation was given from April 1935 to May 1937 before cotton

crop was sown.

The extent of root-rot infection in a particular plot was determined by the number of plants killed by the disease. No regular differences in disease incidence n the treated plots and those continuously cropped with cotton crop were observed. In the 12 months tillage treatment only two plots out of three in 1936 showed some reduction in mortality, whereas the mortality in the third plot was slightly higher than the check plots. In the 24 months tillage treatment also the fall in the incidence of the disease was observed only in two plots. The figures on mortality recorded in 1937 in the treated plots as compared to those in plots continuously cropped with cotton are given below.

Contin	uous crop	ping	24	months	tillage
	54.16			14.07	48.00
	44.06			47.30	
	19.16			17.26	

C. Effect of fertilizers. Experiments to control the disease by direct application of fertilizers to the soil were carried out from 1935 to 1940, and are described, below:

(a) Organic manure.—The beneficial effect of organic manure applied in deep furrows on the incidence of *Phymatotrichum* root-rot of cotton in Arizona in the U.S.A. has been demonstrated by King and his associates [1926, 1929, 1934]. Similar trials were conducted at Lyallpur in 1935 and 1937. In 1937 forty-eight trenches, each 50-ft. long, 12 ft. deep and 1-ft. wide, were dug in a uniformly infected area. These were grouped into eight lots of six trenches each. In each group three trenches were manured about one month prior to sowing cotton, at the rate of 14 tons per acre. In 1935 only 32 trenches were dug and divided into eight lots of four each. Out of these four only two were manured at the rate of 30 tons per acre. The unmanured trenches in each group were refilled with the soil and served as checks. In 1937 American cotton variety 4F and in 1935 Desi cotton variety Mollisoni 15 were sown in the centre of each trench in the middle of May. Observations on mortality due to root-rot disease were made at weekly intervals throughout the cotton season. No regular differences in the incidence of root-rot in the manured and unmanured furrows were observed. The cumulative effect of this treatment on disease incidence was not studied. The data collected in 1937 are given in Table IV.

TABLE IV

Effect of addition of farmyard manure in deep furrows

<i>y</i>	Average per cent disease incidence			
Group	Manured	Unmanured		
I	0·57 0·59 31·20 90·22 85·84 89·75 84·26 93·86	2·48 9·15 73·85 81·32 87·33 88·82 78·18 90·05		

(b) Artificial fertilizers. The effect of a number of fertilizers on disease incidence was tested in uniformly infected fields. Desi cotton variety Mollisoni 15 was sown during the most favourable period for development of the disease. The data of three of some typical experiments are summarized

in Table V, VI and VII. Calcium was applied in three different forms, viz. gypsum, lime-stone or lime, and calcium chloride. The soil at Lyallpur where these experiments were conducted tends to be alkaline, although it gives good yields. In some of the trials gypsum and other calcium compounds were also included, so that the alkalinity of the soil may not interfere with these experiments. These were applied in high doses because some of the work on the reclamation of alkaline soils (by changing sodium clays into calcium clays) which was conducted at the time had shown that in certain localities only high amounts of gypsum and lime, owing to the comparatively low solubility of calcium, have been found to give good results.

In the third experiment (Table VII) the effect of potassium was studied alone and in the presence of calcium for obtaining a clearer comprehension of the results; calcium chloride was used alone as well. These two chemicals were used in the form of chlorides so that there may be no differences in the acid radical and differences in the results, if any, may be directly ascribed to the two types of basic ions.

TABLE V

Effect of fertilizers on disease incidence

No.	Treatment	Rate per acre	Total plants (3 plots)	Plants killed by root-rot	mortality (3 plots)
2 Sup 3 Am ph	monium sulphate perphosphate monium sulphate+superphos- nate to collected from canal beds treated control	85 lb. N. 100 lb. P <sub>3</sub> O <sub>5</sub> 85 lb. N+ 100 lb. P <sub>2</sub> O <sub>5</sub> 73 tons	297 296 294 298 299	75 94 82 6 <b>9</b> 92	25-2 31-7 27-9 23-1 30-8

Table VI

Effect of fertilizers on disease incidence

ī			Average per ce	ent mortality
•	Treatment	Rate per acre	1935-36 (4 plots)	1936-37 (4 plots)
 I	Lime stone	4480 lb. CaO · 200 lb. N. ·	49·5 55·3 36· <b>7</b>	77 · 1 67 · 1 70 · 6
2 3	Farmyard manure Lime stone + F.Y. manure	4480 lb. CaO +200 lb. N. 4480 lb. CaO	48.6	67.8
1 5.	Lime stone+superphosphate  F.Y. manure+ammonium sulphate+superphosphate	+100 lb. PgO <sub>5</sub> 200 lb. N.+ 85 lb. N.+	26-3	50-6
6	Untreated control	100 lb P <sub>2</sub> O <sub>5</sub>	32 - 3	58·X

Table VII
Effect of fertilizers on disease incidence

No.	Treatment	Rate per acre	Average per cent mortality (6 plots)		Yield per 100 plants
				Lb. oz.	Lb. oz.
1 2 3 4	Calcium chloride Potassium chloride Calcium chloride+potassium chloride Untreated control	2281 lb. CaO 210 lb. K. 2281 lb. CaO +210 lb. K.	35·9 31·4 34·8 59·6	894 3 1309 2 1147 10 712 15	9 1 10 11 10 6 9 7

No appreicable reduction in the incidence of root-rot was observed in the plots treated with various chemicals except in the case of plots where calcium chloride and potassium chloride alone and in combination with each other had been applied. The disease incidence in these plots was lower than in the untreated control plots. There were also indications of increased yield where potassium chloride had been applied.

In another experiment in which a still heavier dose of potassium chloride was applied there was no change in the development of root-rot, but the yield of seed cotton per 100 plants was 21 lb. 10 oz. as compared to 15 lb. 12 oz. in the untreated controls.

Varying quantities of gypsum, i.e. from 2 to 6

tons (1456 lb. to 4368 lb. CaO) per acre were also tried in 1938-39. The plots treated with 6 tons of gypsum showed an average per cent root-rot mortality of 57.8 as against 59.5 in the check plots. Lime was applied at the rate of 3 tons (5100 lb. CaO) per acre, but both the disease incidence and vield of seed cotton were unaffected.

In 1937-38 the effect of certain minor elements was studied in a heavily infected field which was divided into sub-plots, 18 ft. × 12 ft. each. Each treatment was replicated five times. *Desi* cotton variety *Mollisoni* 15 was planted on 20 May 1937. Observations on disease incidence were made weekly. Data showing the effect of different treatments on disease incidence and out turn of seed cotton are summarized in Table VIII.

Table VIII

Effect of minor elements on disease incidence

					Average	Average yield of cotton seed		
No.	Treatment	Rate per acre	Total plants	Plants killed	per cent root rot mortality	Per acre	Per 100 plants	
						Lb. oz.	Lb. oz.	
1 2 3 4	Borax (anhydrous) Zinc sulphate Manganous chloride Untreated control	2·15 lb. B. 2·27 lb. Zn. 6·94 lb. Mn.	267 265 284 287	138 155 135 135	$31 \cdot 40$ $46 \cdot 18$ $34 \cdot 71$ $39 \cdot 40$	827 12 607 13 840 11 944 2	16 4 15 5 14 0 15 12	

The elements tested singly failed to exercise any favourable influence on the incidence of the disease and on yield.

In another experiment small plots each 11 ft. × 6 ft., were treated with aluminium sulphate and ferrous sulphate at the rate of 500 lb per acre (149·12 lb. A1<sub>2</sub>O<sub>3</sub> and 262·37 lb. Fe<sub>2</sub>O<sub>3</sub>). Iron and aluminium were not deficient in the soils under these experiments but they were included to study their effect on the incidence of the disease. The plots were andomized and 6 repeats of each treatment were kept. Untreated plots served as check. The observations on disease incidence were made throughout the cotton season. Treated and con-

trol plots did not show any difference in disease incidence but average yield of seed cotton per acre in the treated plots was lower (626 lb.) as compared to the check plots (747 lb.)

pared to the check plots (747 lb.).

Experiments described above conducted with a view to control the root-rot di ease of cetton show that soil fumigation, various cultural treatments and application of fertilizers are of no help in controlling the disease. As already reported [Vasudeva, 1939], even change in soil-reaction failed to reduce the disease incidence. However two simple and most effective methods for the control of the disease in the Punjab, i.e., varying of sowing date and mixed cropping, have already

been described in the preceding numbers of this series [Vasudeva, 1943; 1941].

#### SUMMARY

Results of experiments on soil fumigation, various cultural treatments described in this paper and the application of fertilizers to the soil have not given any indication of the possibility of evolving a practical method of control of rootrot disease of cotton in the Punjab.

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## THE COMBINED ACTION OF ORGANIC MATTER AND PHOSPHATIC FERTILIZERS IN SOILS

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NOTWITHSTANDING the wide variation of soils in different parts of the world, the beneficial action of phosphatic fertilizers when applied in intimate contact with green or other organic manures has been universally recognized. Those who strongly advocate the use of either natural or artificial phosphatic fertilizers for soil amendment always advise that they should be applied in conjunction with decaying organic matter. The explanation usually given for this practice is that organic acids, carbon dioxide, and nitrous acid resulting from the decomposition of organic matter are active agents in making the phosphates available to plants. Strangely, however, attempts made in the laboratory to test this explanation have not given positive results.

It is, however, conceivable that organic phosphates may be produced by the chemical action or through absorption of phosphates with humic or organic acids resulting from the decaying organic matter in the soil. And it is quite probable that such organic phosphates are significantly related to the crop yield resulting from the combined application of phosphatic fertilizers and organic matter.

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The possibility of this effect has not hitherto been sufficiently recognized, and it is to throw light on this aspect of the problem that the present investigation was undertaken.

Recent experience does not justify the assumption that the state of solution is necessary to availability of plant food. As for instance, direct experiments by Truog [1914] and Marais [1922] have shown that the insoluble iron and aluminium phosphates which are believed to be hardly available as plant food, are very well utilized by plants as sources of phosphorus. Therefore, the inevitable conclusion is that solubility is not the dominating factor of availability.

Further, highly dispersed material, though not present in a true solution, can diffuse into plant cells. As for instance, Czapek [1905] states that colloids can enter plant cells, and Pfeffer [1900] records the diffusion of silicic acid into the cell sap of plants. Jennings [1918] has shown that colloidal silica gel can be absorbed by wheat seedlings in water culture solutions.

In the light of the above observations Comber [1922] developed a hypothesis as to the availability of mineral plant food in soils. According

to him, food materials in the colloidal state are easily taken up by plants and the cell sap of the root hair possibly derives its food material without any acid secretion as formerly supposed. As for example, ferric phosphate loses its colloidal properties on ignition, as a result of which it becomes unavailable to the plant according to Prianischnikow [1905]. On the other hand, aluminium phosphate retains its colloidal properties after ignition and remains available for the plant. Thus, the colloidal condition of phosphates is in a large measure related to their solubility and their availability to the plant.

Now if it can be demonstrated that organic phosphates resulting from the action of fermenting organic matter on phosphatic fertilizers possess colloidal properties, remain in the soil in a more dispersed state than the inorganic phosphates used as fertilizers, and eventually become more available to plants, then the increased crop yield obtained from phosphatés when applied to soils in conjunction with organic matter may be explained to have originated from the colloidal organic phosphates formed.

In order to test this, composting experiments were instituted with lucerne as a typical source of organic matter. Monocalcium phosphate, the chief phosphatic constituent of superphosphate, and dicalcium phosphate were used in the following proportions for making composts with lucerne:

1) 70 lb. of lucerne alone

(2) 60 lb. of lucerne + 237.32 gm. of monocalcium phos-

phate (= $\frac{1}{4}$  lb. P<sub>2</sub>O<sub>5</sub>) (3) 60 lb. of lucerne +257.50 gm. of dicalcium phosphate (= $\frac{1}{4}$  lb.  $P_2O_5$ ).

It took about four weeks to complete the decomposition of lucerne. When the whole mass was homogeneous, the stuff was dried in the sun and sampled. The chemical composition of the composts is given in Table I.

TABLE I Chemical composition of composts

			By H	HCI	Per cent soluble P2O5 by		
No.	Kind of composts	N per cent	$\begin{array}{c} \text{Total} \\ \text{P}_2\text{O}_5 \\ \text{per cent} \end{array}$	$K_2O$ per cent	I per cent citric acid	1 per cent K <sub>2</sub> CO <sub>3</sub>	
1	Lucerne alone	2.712	1.509	7 · 898	1.032	$0 \cdot 355$	
2	Lucerne+monocalcium phosphate .	2.717	4.861	7 · 805	1.952	0.831	
3	Lucerne+dicalcium phosphate	2.576	$4 \cdot 279$	6.683	2.076	1 · 157	

TABLE II Balance sheet of P<sub>2</sub>O<sub>5</sub> in composts

		Lb. $P_2O_5$ in compost			Lb. P <sub>2</sub> O <sub>5</sub> extracted by		
No.	Kind of composts	HCl Sol. P <sub>2</sub> O <sub>5</sub> in lucerne	$\begin{array}{c} \text{Added} \\ \text{P}_2\text{O}_5 \end{array}$	$\begin{array}{c} \operatorname{Total} \\ \operatorname{P}_2\operatorname{O}_5 \end{array}$	HCl	l percent citric acid	1 percent K <sub>2</sub> CO <sub>3</sub>
1 2 3	Lucerne alone	0·0815 0·0739 0·0739	nil 0 · 2500 0 · 2500	0·0815 0·3239 0·3239	0·0815 0·2625° 0·2311	0·0557 0·1054 0·1121	0·0193 0·0449 0·0625

	Per cent of HCl sol	uble $P_2O_5$ extracted by	Per cent of total P2O5 extracted by		
	I per cent citric acid	1 per cent K <sub>2</sub> CO <sub>3</sub>	HCl	1 per cent citric acid	1 per cent K <sub>2</sub> CO <sub>3</sub>
$\begin{bmatrix} 1 \\ 2 \\ 3 \end{bmatrix}$	68·39 40·16 48·52	23·66 17·10 27·04	100 81·04 71·34	68·39 32·54 34·61	23·66 13·86 19·29

It will be noticed that, although the same amount of  $P_2O_5$  was originally present while making the last two composts, the final products appreciably differ in composition in their relation to  $P_2O_5$  content. This is shown by the different proportions of  $P_2O_5$  extracted with the various solvents, e.g. hydrochloric acid, I per cent citric acid of Dyer [1894] and I per cent potassium carbonate solution of Das [1930]. This effect may be further examined by drawing up the balance sheet of  $P_2O_5$  in the composts as shown in Table II. Each compost had a dry weight of 5.4 lb.

The indefinite composition of the various composts is clearly reflected in the different solubilities of their P<sub>2</sub>O<sub>5</sub> content in the solvents tried. The phosphate composts may still, however, contain some of the added inorganic phosphates unacted upon by the fermenting organic matter; this may partly account for the different amounts of P<sub>2</sub>O<sub>5</sub> extracted. With a view to eliminate this possibility and in order to obtain more rigorous proof as to the variable nature of the composts, humus was extracted from the individual composts in the following manner and P<sub>2</sub>O<sub>5</sub> determined in the same for comparison.

Composts were shaken for six hours in the cold with one per cent potassium carbonate solution or water alone in the ratio of 10 gm. of substance to 100 c.c. of the solvent and allowed to stand overnight. To the deep brown filtrates hydrochloric acid was added in slight excess, which precipitated humus keeping other substances in solution. The humus was filtered and washed free from hydrochloride acid with water and dried at 100°C. The results of P<sub>2</sub>O<sub>5</sub> estimations are given in Table III.

TABLE III

P<sub>2</sub>O<sub>5</sub> content of humus extracted from composts with water or 1 per cent potassium carbonate solution

Kind of composts -	Per cent P <sub>2</sub> O <sub>5</sub> in humus extracted by			
	Water	K <sub>2</sub> CO <sub>3</sub>		
1. Lucerne alone 2. Lucerne+monocalcium	0.8542 0.8859	0.3582 0.6229		
phosphate 3. Lucerne + dicalcium phosphate	0.7741	0.4509		

It is evident that the P<sub>2</sub>O<sub>5</sub> content of humus obtained from the three sources is different, and consequently the proportion in which fermenting organic matter combines with phosphates is not constant. The nature of these compounds will be better intelligible in the light of the following considerations.

It is well known that humus precipitated by acids is different from humus not so precipitated, both of which play an important role in soil phenomena. They are not definite in composition, consisting of several complex compounds, the nature of which depends on the character of the sources from which they are derived. On carefully examining a number of soils in their vegetation relationships it has been shown that there must be several distinct types of humus, which, however, cannot be classified, according to Russell [1921], for lack of sensitive laboratory methods.

Consequently, some of the organic compounds which are normally present in humus, no doubt, combine by means of adsorption or chemical action with phosphates naturally occurring in lucerne or artificially added as fertilizers, and the complex organic phosphates thus formed, which may be either chemical compounds or adsorption complexes or mixture of both, may not therefore have the same composition. A discussion as to the probable constitution of these complex bodies evidently lies beyond the scope of the present investigation. It was, however, considered essential that these organic phosphates should be isolated, qualitatively at least, to study their suitability as plant food in relation to soil fertility.

In order to do this, individual composts were shaken with water for six hours in the proportion of 10 gm. cf substance to 100 c.c. of water and allowed to stand overnight. The deep brown filtrates were then dialysed through parchment paper. None of the brown liquid contained within the parchment bag diffused into outside water, which was changed every day till no trace of  $P_2O_5$  nor the presence of any salt could be detected in it. The complex organic phosphates thus isolated in solution behaved like colloids and did not settle down even on long standing. The results of their  $P_2O_5$  determination are set forth in Table IV.

 $\begin{array}{cccc} & \text{TABLE IV} \\ P_2O_5 & \text{content of dialysed solutions of complex} \\ & \text{organic phosphates of composts} \end{array}.$ 

Complex organic phosphates from composts of	Time taken for dialysis	Mg. P <sub>2</sub> O <sub>5</sub> per 100 c.c.
1. Lucerne alone 2. Lucerne+monocalcium	20 days 16 ,,	3·1 10·2
phosphate 3. Lucerne+dicalcium phosphate	23 "	4.9

It is evident that these complex organic phosphates from the three sources are different in constitution with respect to their P<sub>2</sub>O<sub>5</sub> content.

Next, in order to test how far these colloidal phosphates could be utilized for supplying the

phosphatic needs of plants, Knopp's nutrient solution was prepared having the following com. position for water culture experiments:

 $Ca(NO_3)_2 \dots$   $MgSO_4$ ,  $7 H_2O \dots$ 4 gm. FeC1 ..... Trace KNO3 ..... 1 gm. K<sub>2</sub>HPO<sub>4</sub> .....  $1 \text{ gm.} (=0.4081 \text{ gm.} P_2O_5)$ KC1 ..... 0.5 gm.

All the potassium salts and the rest are dissolved separately; the two solutions are then mixed together, diluted, and the volume made up to seven litres with water. The strength of the final solution is 0.011 per cent of salts. On allowing to stand, a little tricalcium phosphate is precipitated which lessens the proportion of calcium and phosphoric acid in the solution. The P<sub>2</sub>O<sub>5</sub> was found reduced to 0.043 gm. per litre against 0.0583 gm. in the original solution.

As a preliminary study, a series of water culture experiments was instituted with seedlings of ragi (Eleusine coracana) in order to test the efficiency of the colloidal organic phosphates against K2HPO4 in the Knopp's nutrient solution. The seedlings

of about the same size and weight were placed in all the solutions. Loss of water was made up by the addition of water every day. The following solutions were used:

(1) 1200 c.c. of Knopp's nutrient solution;

this served as the control

(2) Equivalent Kncpp's solution devoid of P2O5 plus equivalent P2O5 (as present in Knopp's solution) in the form of colloidal organic phosphates from the compost of lucerne and monocalcium phosphate

(3) Equivalent Knopp's solution devoid of P<sub>2</sub> O<sub>5</sub> plus equivalent P<sub>2</sub> O<sub>5</sub> as colloidal organic phosphates from the compost of lucerne and dicalcium phosphate

(4) Equivalent Knopp's solution devoid of P<sub>2</sub> O<sub>5</sub> plus equivalent P<sub>2</sub> O<sub>5</sub> as colloidal organic phosphates from the compost of

lucerne alone.

In this study no account was taken of the small variations in the ionic ratios of the different culture solutions evidently brought about by the presence of organic matter in the colloidal organic Observations made qualitatively phosphates. were recorded for a period of 24 days. It was found that the growth of seedlings in solutions (3) and (4) was superior to that in the control (1), whereas the growth in (2) was inferior. The colour of the latter solution was much deeper than that of (3) and (4).

For further study the solution (2) was diluted to half the strength with respect to P<sub>2</sub>O<sub>5</sub> content by adding 1200 c.c. of the Knopp's solution devoid of  $P_2O_5$ . The colour of the resultant solution became lighter and almost of the same depth as in (3) and (4). The solution was placed in two vessels, 1200 c.c. in each, and the experiments were repeated with 12 seedlings of about the same size and weight in each along with the other culture solutions previously used. Observations were recorded for a period of five weeks.

The initial and the final weights of the seedlings were recorded as set forth in Table V. The differ-

ential growth of the seedlings could very well be visualized in the control and the rest of the nut-

rient solutions.

TABLE V

The growth of ragi seedlings in nutrient solutions having inorganic and organic phosphates in them separatelu

No.	Nature of nutrient solutions	Weight in mg. of 12 seedlings			Per cent increase
		Initial	Final (After 5 weeks)	Difference	over control
1 2	Knopp's solution (Control) Knopp's solution devoid of $P_2O_5+$ organic phosphates	4·4	10.2	5 · 8	
	from the compost of lucerne+monocalcium phos-		17.0		0.1.4
	phate	$egin{array}{c} 4\cdot 1 \ 4\cdot 3 \end{array}$	$\begin{array}{c c} 15 \cdot 2 \\ 15 \cdot 5 \end{array}$	$\begin{array}{c c} 11 \cdot 1 \\ 11 \cdot 2 \end{array}$	91 · 4 93 · 1 <sup>4</sup>
3	Duplicate.— Do. Do Do. + organic phosphates from the compost of	4.3	19.9	11-2	93.1
0	lucerne+ dicalcium phosphate	4.2	14.8	10.6	82.8
. 4	Do. + organic phosphates from the compost of				
	lucerne alone	4.3	14.5	10.2	76.0

It is evident that the duplicate experiments with solution (2) agreed well among themselves and the seedlings in them were somewhat better than those in solutions (3) and (4). In all the solutions (2), (3), and (4) the growth of ragi seedlings was practically of the same order and decidedly better than that in solution (1) which served as the The percentage increases of growth indicate clearly the superiority of colloidal organic phosphates over K<sub>2</sub>HPO<sub>4</sub> present in Knopp's solution. With regard to the efficacy of organic phosphates derived from different sources it is found that the compost of lucerne and monocalcium phosphates is the best, next comes in order the compost of lucerne and dicalcium phosphate, and the last is the compost of lucerne alone. In actual field practice also it is observed that the composts of green manure and superphosphate or both ploughed in together always give better crop yields than either superphosphate or green manure applied alone.

The conclusion therefore emerges that colloidal organic phosphates can very well maintain the growth of seedlings and serve as better agents for supplying the phosphatic requirements of crops than inorganic phosphates present in Knopp's solution, or similar phosphates normally contained in the phosphatic fertilizers used in ordinary farm practice. Probably the presence of colloidal organic matter in these solutions helps the assimilation by plants of other food materials too -unlike the same in Knopp's solution-according to the hypothesis already described, by making colloidal contact of root-hairs with food materials.

Hutchinson [1923] has shown by field experiments that the application of previously fermented green-manure, sunn-hemp (Crotalaria juncea) with superphosphate gives greater crop returns in calcareous Pusa soils than the same ploughed in along with super. The green-manure combined with super, on the other hand, yields better crops than super alone. The advantage of using previously fermented green-manure obviously lies in the fact that the humus produced by fermentation can at once react with super which otherwise becomes reverted into insoluble phosphates on reacting with soil bases, rendering the formation of colloidal organic phosphates rather a slow process.

The recent work of Spencer and Stewart [1934] lends support to the contention as to the better efficiency of organic phosphates in soil. They showed that phosphates in organic form of the type formula R(OH)x (OPO3My)z escapes, to a marked degree, the fixation in soil which normally occurs to the phosphates applied in some inorganic forms. They experimented with organic phosphates of the above type prepared in the laboratory, such as,

calcium mono-orthophosphate of glycerol  $C_3H_5$ potassium sorbitvl and (OH)<sub>2</sub>OPO<sub>3</sub>Ca  $orthophosphate \ C_6H_8(OH)_4(OPO_3K_2)_2.$ A highly calcareous soil did permit little phosphorus in the filtrate when a solution of pure monocalcium phosphate was allowed to percolate through it, whereas, under the same conditions, much phosphorus passed through the soil when the above organic phosphates were used for percolation. Thus the permeation of phosphate in the organic form into deeper soil layers in close environs of plant roots is assured. This is also supported by the theory put forward by Ramann [1911] and Comber [1922] which suggests a distinct possibility of the plant roots absorbing phosphate direct, probably through contact with the soil colloids and also by the hypothesis of Greenhill [1930], according to which, 'solid phase' feeding of phosphoric acid by the roots of crops is probable.

## SUMMARY AND GENERAL CONCLIUSIONS

1. Organic phosphorus complexes are formed in the soil by the combined action of decaying organic matter and phosphatic fertilizers. They were qualitatively isolated by dialysis from the compost of lucerne alone, and also from lucerne composted with monocalcium and dicalcium phosphates separately.

2. These phosphates are soluble in water, neutral in reaction, and possess colloidal properties. They remain in a highly dispersed state in the soil and have been shown to be more available to plants by water culture experiments than the inorganic phosphates of phosphatic fertilizers used in ordinary farm practice, which are generally insoluble or eventually become so on reacting with soil bases.

3. The beneficial action of these colloidal organic phosphates resulting from the combined application of phosphatic fertilizers with organic manures in soils is explained on the basis of certain hypotheses mentioned in the paper, according to which, colloidal substances can serve as plant foods and the direct dissolution of plant food is rendered possible by the cell sap of root hairs.

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### AVAILABILITY OF SUPERPHOSPHATE WITH DEPTH OF ITS PLACEMENT IN CALCAREOUS SOILS

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THE past researches and the earlier work of Das [1921] in this laboratory Harrison and show that the soluble phosphoric acid of superphosphate is retained in the surface layers of calcareous soils as insoluble calcium phosphates by chemical combination with the large amount of calcium carbonate normally present in them; in consequence, the action of super is very localized, resulting in an uncertain and disappointing crop response to superphosphate in calcareous soils. Another contributory factor to this effect is due to the presence of 50 to 60 per cent of gypsum in commercial super, whose depressing action on the cropping of calcareous soils has already been shown by the author [Das, 1933].

Hockensmith, et al. [1933] studied the movement of phosphate in a calcareous soil by mixing a phosphate fertilizer with the surface 2 in. of soil in 5-gallon jars, and found the amount of phosphate leached through the soil to be They also determined the exceedingly small. effect of depth of placement on the availability superphosphate in a calcareous soil by applying it at various depths in 5-gallon jars and found that the depth of application made a marked difference in the yield of alfalfa.

Midgley [1931] found that applications of super 6 in. deep gave 57 and 86 per cent more yields of grass and weeds respectively than the surface application. Similarly blue grass gave 46, 166 and 149 per cent more yields by surface 3 in. and 6 in. deep applications of super than no treatment of super. In the case of corn, sorghum and Sudan grass 110 to 167 per cent greater increases over control were obtained by 3 in. and 6 in. deep applications of super. He also found that under field conditions 60 to 100 lb. of  $P_2O_5$  per acre as super applied on the surface are retained within the surface inch even after an interval of six months,

As the surface application of the fertilizer is a common farm practice, it appears to be a serious obstacle in the way of an extensive penetration of the soluble phosphoric acid of superphosphate into calcareous soils. It was, therefore, considered worthwhile to discover some means which would render a more extensive permeation of the phosphate into the soil below the plough line and thus result in a greater proximity of the applied phosphate to the plant's zone of absorption.

Although calcareous soils round about Pusa belonging to the Indo-Gangetic alluvium and containing 30 to 40 per cent of chalk are particularly deficient in available phosphates, their response to superphosphate is often erratic owing to the operation of factors discussed above. The surface application of the fertilizer which is usually followed in ordinary farm practice, accounts for this peculiar behaviour to a certain extent. Should, therefore, superphosphate be applied at different depths of such a soil, a variation in response is expected, depending on whether the crop grown is a shallow-rooted or a deep-rooted one; this observation may also throw considerable light in elucidating the problem of manuring these calcareous soils, particularly with superphosphate.

With this object in view, the present investigation was undertaken.

A sample of calcareous Pusa soil containing about 33 per cent of calcium carbonate was collected from a fallow plot for pot experiments in 1933. Four pots of similar dimensions, e.g. 12 in. in diameter and about 22½ in. high formed a group and received similar treatment. The pots contained 50 kilos of air-dry soil in each. A basal dressing of potash and nitrogen was given to all the pots at the rate of 80 and 100 lb. per acre or 40 and 50 mg. per kilo of soil as sulphates of potassium and ammonium respectively. Phosphate (P<sub>2</sub>O<sub>5</sub>) was applied to different

groups of pots at 100 lb. per acre or 50 mg. per kilo of soil as superphosphate at different depths, e.g. at surface, and 4, 8, 12, and 16 in. below the surface. The pots were watered from the top to get different degrees of diffusion, if any, and 16 per cent of moisture was maintained in the soil

throughout the experiment. Mustard was sown on 8 November 1933 and the crop harvested on 10 March 1934.

Although the weights of both grain and straw per pot were recorded separately, only the yield of grain is given in Table I.

Table 1

The yield of mustard with the placement of superphosphate

	Replications		Mean	Percent-		
Treatment	1	2	3	4	yield in gm.	crease over surface applica- tion
Super at surface  , 4 in. below surface , 8 in. below ,, , 12 ir. below ,, , 16 in. below ,,	29·6 44·5 35·9 41·3 37·1	28·8 45·2 35·1 36·3 36·2	31·2 37·6 38·9 33·2 30·0	23·6 41·9 34·5 31·4 31·8	28·30 42·30 36·10 35·55 33·78	49·5 27·6 25·6 19·4

Standard error for comparison of mean yields by Fisher's [1932] analysis of variance = 2.386

Critical difference for P=1 per cent =  $7 \cdot 03$ Critical difference for P=5 per cent =  $5 \cdot 09$ 

From Table I it is evident that all the deeper treatments of super have produced 19 to 50 percent higher yields of mustard than the surface application. Further, the differences in mean yields of grain between surface and every other treatment of super are highly significant, being greater than the critical value of difference for one per cent level of significance in every case except the 16 in. application, where holds 5 per cent level of significance. Super placed 4 in. deep produced the maximum crop and gave highly significant yield when compared with any of the treatment, tried. Among the last three treatments there is no significant difference of mean yields.

Mustard being a shallow-rooted crop, appears to derive for maximum crop production its requisite phosphatic nutrition from the soil where super

has been placed 4 in. deep. After the harvest of mustard crop was over, 4 in. soil borings of 20 in. deep soil from each of the differently treated groups of pots and also from a pot of untreated Pusa soil which served as the control for comparison were taken from the surface As these pots were watered for downward. about four months during the period of growing the mustard crop, the super applied to the pots These soil could leach downward, if possible. samples were examined for total phosphate in order to discover the progressively downward leaching of the phosphate, if any. The results obtained are given in Table II.

TABLE II

Total phosphate contents of 4 in. soil borings from pots where superphosphate was applied to different depths at the rate of 50 mg. of  $P_2O_5$  per kilo of soil and also from the control pot of untreated Pusa soil

Treatment	Mg. total P <sub>2</sub> O <sub>6</sub> per 100 gm. of soil				
Trestment	0-4 in.	4-8 in.	8-12 in.	12-16 in.	16-20 in.
Control	- 104 - 110 - 101 - 104 - 104 - 104	104 101 107 104 104 199	104 101 99 109 104	100 104 104 101 115 99	104 104 104 104 100 107

The figures in italics in Table II show the higher concentrations of P<sub>2</sub>O<sub>5</sub> in the depths where super has been applied. At the other depths the concentration of P2O5 is almost of the same magnitude as in the corresponding depths of the control where no super has been applied. In other words, it never exceeds the limit of P2O5 content in any depth of the control pot. The conclusion therefore emerges that phosphate does not leach downward beyond 4 in. from the depth of its application, but remains within the 4 in. area of the depth of its placement in the

The foregoing cropping results were secured with a rabi (winter) crop. Next it was considered worthwhile to obtain further confirmatory data with a kharif (summer) crop. For this purpose pot experiments were conducted on similar lines in 1934 with the same calcareous Pusa soil. Super was placed at 3, 6, 9 and 12 in. below the surface soil. Ragi (Eleusine coracana) was sown on 20 June and the crop harvested on 5 October, 1934. The yield of grain of individual pots is set forth in Table III.

From Table III it is evident that the differences in mean yields of grain between the surface appli-

TABLE III The yield of ragi (Eleusine coracana) with the placement of super

${f Treatment}$	Replications				Mean	Percent- age in-
Treatment	1	2	3	4	yield in. gm.	crease or decrease over surface application
Super at surface	48·7 40·4 70·6 59·7 44·3	47.6 34.9 63.9 63.2 46.0	44·9 33·9 66·6 60·1 43·9	46·1 31.4 68·4 61·1 42·2	$46 \cdot 83 \\ 35 \cdot 15 \\ 67 \cdot 38 \\ 61 \cdot 28 \\ 44 \cdot 10$	$\begin{array}{c} -25 \cdot 0 \\ +44 \cdot 0 \\ +30 \cdot 9 \\ -5 \cdot 8 \end{array}$

Standard error for comparison of mean yields by Fisher's [1932] analysis of variance = 1.784 Critical difference for P=1 per cent=5.26 Critical difference for P=5 per cent=3.80

cation of super and super placed only 6 or 9 in. deep are highly significant being greater than the critical value of difference even for one per cent level of significance. On the other hand, such differences in mean yields of grain between the surface and 3 in. or 12 in. deep treatment of super are negative, and consequently no profitable response can be expected on the application of super at these depths for a kharif crop like ragi. Super placed 6 in. deep produced the maximum crop and gave highly significant yield when compared with any of the treatments tried. Next came in order the yield from the 9 in. deep application of super.

Thus, the cropping experiments with a kharif crop like ragi and a rabi crop like mustard have shown that the application of superphosphate 4 in. to 6 in. below the surface gives the maximum crop production in calcareous soils. This may be easily effected by means of deep ploughing in ordinary farm practice.

#### SUMMARY AND GENERAL CONCLUSIONS

The present investigation was instituted to discover some means which would render a more extensive permeation of the phosphate into the soil below the plough line and thus result in a

greater proximity of the applied fertilizer to the plant's zone of absorption. In order to do this, superphosphate was applied at different depths of a calcareous Pusa soil in two series of pots, in one of which mustard, a rabi (winter) crop and in the other ragi (Eleusine coracana), a kharif (summer) crop were grown. The cropping results showed that the application of super from 4 in. to 6 in. deep gave the maximum crop production.

After the harvest of mustard crop was over, 4 in. soil borings from surface downward of the different groups of pots where superphosphate was applied at surface, and 4, 8, 12, and 16 in. deep were examined for total phosphate contents. The results showed that phosphorus did not leach downward beyond 4 in. from the depth of application, but remained within the 4 in. area of the depth of its placement in the soil. This is fully in agreement with the observation of other workers.

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# CONDUCTOMETRIC METHOD OF ANALYSIS AS APPLIED TO SOIL SURVEY WORK

# III. THE ESTIMATION OF THE SOLUBLE SULPHATE AND CHLORIDE CONTENTS OF SOILS\*

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(With one text-figure)

THE soils from areas subjected to arid climatic conditions contain soluble salts in varying degrees. The main salts present in the alluvial soils of the Punjab are sulphate and chloride of sodium. For characterizing such soils it is necessary to determine the total soluble salts and at times the exact proportion of the main salts present. The analyses are usually done with water extracts of soils which are prepared from soil suspensions of definite soil-water ratio (one part of soil and five parts of distilled water are usually employed) after shaking them for some time and extracting the suspensions with Houston pump. The chlorides are estimated volumetrically and, unless the soil extracts are coloured, that work does not present much difficulty. The usual method of estimating sulphates gravimetrically is, however, fairly tedius and time consuming. A quicker method for such analysis and capable of use in the field would be extremely helpful for workers engaged in soil survey work. The present paper describes a method based on the conductometric principles for such analysis.

#### PREVIOUS WORK

Britton [1934] describes a number of methods used for conductometric analysis. The majority of these are based on the use of alternating current in the equipment to avoid polarization effects. The oscillating thermionic valve is most commonly employed, the advantage being that the frequency of the A.C. can be varied at will to suit experimental requirements. Callan and Horrobin [1928] used the carborundum crystal detector in place of the thermionic valve in the circuit, thus simplifying the equipment for conductometric analysis considerably. Following their suggestion, Hoon [1932] contrived a simple equipment for the conductometric estimation of sulphate and chloride contents of soils. Although the method was fairly quick and simple it was primarily suited for use in the laboratories or places where electric current was handy to drive the commutating arrangement employed therein.

Some equipments for conductometric analysis involve the use of direct current. Puri and Anand [1937] suggested an equipment for determining the salt content of soil suspensions in which a high tension dry battery was employed for direct current. The Dionic Water Tester, another convenient outfit present in almost every soil laboratory for measuring the salt content of solutions, water samples, etc., also involves the use of direct current. Hoon, Malhoutra and Jain [1939] adopted Dionic Water Tester for determining the percentage salt centent of soils.

#### THE USE OF THE DIONIC WATER TESTER

As the Dionic Water Tester yields an almost constant potential of about 100±0.25 volts for a few seconds it was considered of interest to examine the possibility of employing this instrument for the conductometric titration work. Rae [1931] employed the Dionic Water Tester for titrating acids and alkalies by taking inconvenient volumes of the two solutions of decinormal strength. No reference, however, had come to our notice of the use of this apparatus for titration of solutions involving precipitation of any of the products of reaction. As this apparatus had been adopted for the determination of the percentage soluble salt contents of soils an attempt was made to see if it could also be used for the conductometric titration of the sulphates and chlorides in the water extract of soils.

#### DETAILS OF THE PRESENT METHOD

(a) Equipment

The main requirements are the conductivity meter, the arrangement of movable and fixed electrode complete with flexible lead but without the outer glass container and a wide mouthed bottle of, say, four ounces capacity to serve as a vessel for carrying the titration. Bottles Nos. 2410 and 2412 (A. Gallenkamp & Co., Ltd: Catalogue for laboratory apparatus and equipment, 11th Edition) are very convenient for the latter purpose. Somewhat bigger length of the movable electrode, than the one originally fitted in the standard form, was employed. This simple alteration was found very helpful in as much as it made possible the titration of even solutions with

<sup>\*</sup> For Parts I and II of this series please refer to serial numbers 3 and 4 of the references given at the end of the paper

very low conductivities. A standard length of the electrode or of the glass separator tube was not necessary for our persent work as we required only comparative and not absolute values of conductivities.

# (b) Titration technique

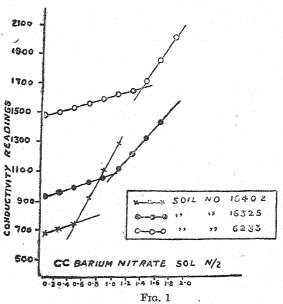
A known volume of the soil extract is taken in the wide-mouthed bottle and neutralized with standard HCl using methyl orange as indicator to decompose carbonates, bicarbonates, etc. avoid the dilution effect, the initial quantity of the extract is so chosen as not to use more than 2 c.c. of the precipitant. A preliminary qualitative test with barium chloride solution is found helpful in deciding the point. The volume is made to 21100 100 c.c. with distilled water and its conductivity noted. Seminormal solution of barium nitrate is then added from a small sized burette, reading preferably to 0.05 c.c., in lots of 0.2 c.c. or less each time. After each addition of precipitant the bottle is stirred vigorously to precipitate out barium sulphate as completely as possible and the conductivity of the solution is noted after shaking each time till a constant value is obtained. In the course of the titration a stage is invariably reached when the conductivity suddenly shoots up indicating that the whole of the sulphate in the solution has been completely precipitated and free barium nitrate is present in excess. When this point is reached two or three further small additions of the precipitant are made and the conductivity of the solution noted after each addition. The conductivity values noted during the titration are plotted against the volume of the precipitant added each time. The curve thus obtained manifests invariably a kink which corresponds to the end point of the reaction.

The conductometric estimation of the chloride content of the soil extracts is done on lines similar to those described for estimation of sulphates except that the initial neutralization of the alkalinity of the extract is done with standard nitricacid or sulphuric acid and the standard solution of silver nitrate is employed as the precipitant. As the precipitation of silver chloride in this case is immediate no vigorous shaking, as required in the case of sulphate titration, is necessary.

# GRAPHIC REPRESENTATION OF THE RESULTS

Using the present tochnique curves were obtained for the titration of standard solutions of sodium sulphate and sodium chloride with barium nitrate and silver nitrate respectively. These were analogous to those obtained by other methods. The results of titration of sulphates in soil

extracts are graphically represented in Fig. 1, and show that kinks representing the end points are clearly obtained.



A curious difference was noticed in the nature of the titration curves obtained with pure salt solutions and soil extracts. While in the case of the former there is no change in conductivity until the end point, in the case of the latter the conductivity changes constantly. The explanation that can be offered for this discrepancy at the present stage of this investigation is that it is due to some peculiar soil factor affecting the extract. A strong support is lent to this view by the observation that sodium sulphate when added to a soil extract containing initially little sodium sulphate gives a titration curve analogous to that of soil extract given in Fig. 1. The exact nature of the soil constituent causing this discrepancy is under investigation.

To compare the results of sulphates in the water extracts of soils the estimations were done for a number of soils both conductometrically and gravimetrically. The results are given in Table I and comparison between the percentage sulphate content as determined by the two methods is very good.

# BARIUM ACETATE SOLUTION AS PRECIPITANT

Britton [1934] suggests the use of the barium acetate solution as the precipitant for the conductometric titration of sulphates. It was tried with the present technique also. The curves obtained were similar to those obtained by other

TABLE I

A comparison of the sodium sulphate	in the water
extracts of soil samples estimated	gravimen i-
cally and conductometrica	illy

Sr. No.	Percentago gm. of Na <sub>2</sub> SO <sub>4</sub> es- timated gra- vimetrically	Percentage gm Na <sub>2</sub> SO <sub>4</sub> estimated con- ductive tically
	0.06	0.07
$egin{smallmatrix} 1 \\ 2 \end{bmatrix}$	0.06	0.07
3	0.09	0.10
4	0.09	0.10
5	0.14	0·14 0·19
6	0·18 0·14	0.13
7 8	0.15	0.12
ŷ	0.11	0.13
10	0.13	0.11
11	0.19	0.15
12	0·13 0·17	0.18
13 14	0.14	0 12
15	0.16	0.18
16	0.19	0.18
17	0.19	$0.18 \\ 0.19$
18	$\begin{array}{c} 0 \cdot 20 \\ 0 \cdot 11 \end{array}$	0.11
19 20	0.15	0 15
21	0.25	0.23
$\overline{22}$	0.28	0·27 0·25
23	$\begin{array}{c} 0.24 \\ 0.22 \end{array}$	0.20
$\begin{array}{c} 24 \\ 25 \end{array}$	0.26	0.25
26	0.27	0.26
27	0.27	0·28 0·32
28	0·29 0·21	0.18
29 30	0.23	0.23
31	0.28	0.28
32	0.21	$0.22 \\ 0.32$
33	$0.29 \\ 0.21$	0.22
34 35	$0.\overline{22}$	0.24
36	. 0.29	0.28
37	0.39	0.37
38	0.36	0·34 0·32
39 - 40	0·33 0·37	0.35
40	0.31	0.31
42	.   0.34	0.36
43	0.30	$0.29 \\ 0.32$
44	0·30 0·37	0.40
$\begin{array}{c} 45 \\ 46 \end{array}$	0.38	0.37
47	0.37	0.36
48	0.40	0·39 0·50
49	0·49 0·43	0.38
50 51	0.43	0.46
52	0.43	0.46
53	0.23	0.51
54	0.54	0·56 0·52
55 56	0:50 0:58	0.55

TABLE I-contd.

Sr. No.	Percentage gm. of Na <sub>2</sub> SO <sub>4</sub> estimated gra- vimetrically	Percentage gm. of Na <sub>2</sub> SO <sub>4</sub> estimated con- ductivetically
58	0.61	0.61
59	0.66	0.65
60	0.64	0.60
61	0.62	0.61
62	0.65	0.64
63	0.80	0.82
64	0.98	0.82
65	0.97	0.89
66	1.03	0.95
67	1.33	1.49
68	1.23	1.28
69	1.44	1.37
70	1.39	1.34
71	1.05	1.01
72	1.35	1.31
73	2.90	2.84
74	2.84	2.76
75	2.91	3.01
76	2.44	2.33
77	3.12	3.05

methods. Here, due to the low mobility of CH<sub>3</sub>COO' ion, the angle of intersection between the two parts of the curve is smaller and thus the indication of the end point is clearer than in the case of barium nitrate.

#### SUMMARY

A technique of conductometric titration of sulphates and chlorides in the water extracts of soils based on the use of the Dionic Water Tester equipment has been described. The method is extremely simple and the outstanding advantage is that it does not require the use of any electric mains, battery, etc. and, therefore, can be used both in the laboratory and the field.

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# STUDIES ON THE FALSE-SMUT DISEASE OF PADDY CAUSED BY USTILAGINOIDEA VIRENS (CKE.) TAK.

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THE false-smut disease of paddy caused by Ustilaginoidea virens (Cke.) Tak. occurs all over Burma but ordinarily does not cause any appreciable damage sufficient to warrant treatment. In the year 1935 the disease appeared in a serious epidemic form at Hmawbi and the surrounding tracts. Elsewhere the disease was sporadic and the loss negligible except at the Agricultural Sta-

tion, Akyab.

At Hmawbi, where detailed investigations were carried out, it was found that the chief centre of infection lay in the Agricultural Farm where, in some of the plots, not a single plant free from the disease was found and the number of grains infected in these plots amounted to about six per cent. In the surrounding cultivators' areas the intensity of the disease was slightly less but farther away it was practically negligible.

In the year 1936 the disease again appeared but in a milder form and in 1937 the amount of infection was extremely light. From 1938 to

1941 the disease did not cause any anxiety.

#### DESCRIPTION OF THE DISEASE

The fungus attacks the ovaries and transforms them into large roundish green masses which may be about twice the diameter of normal grains. The glumes remain unaffected. In each panicle only a few grains irregularly distributed, are usually attacked. On cutting a cross section of an affected grain, it is seen that the central portion consists of a white compact mass bounded by an orange-yellow zone and surrounded by a powdery

dark green layer.

The young ovary is attacked by the fungus at an early stage of its development and in 10 to 15 days after the opening of the flower it is transformed into a hard sclerotial mass covered over with a green powder which consists of spores. The centre of this structure is composed of a pseudo-parenchymatous tissue but towards the periphery the hyphae have a radial arrangement. The spores are formed laterally on these radial hyphae. The youngest spores are almost colourless and are found on the hyphae near the base. Farther out the spores are orange-yellow in colour and near the surface, where they are quite mature, they are dark olive green. The fully formed spores are spherical with a granular coating. They measure from 4 to 6  $\mu$  in diameter.

CULTURAL STUDIES

Germination of the spores

Fresh sclerotial bodies were obtained from the farms at Hmawbi and Mandalay for the study of the germination of spores. The spores were transferred by means of a sterile needle into a tube containing melted plain agar medium at 45°C. The tube was then well shaken and the contents poured into a petri-dish and stored at laboratory

temperature.

At the end of 24 hr. when the dishes were examined it was found that about 10 per cent of the spores had germinated each producing a short unbranched germ-tube. After another 48 hr., the germ tubes were found, profusely branched and septate and bearing clusters of small pearshaped hyaline conidia at the tips. These secondary conidia were formed both terminally and laterally at the hyphal tips. The germination of the spores was limited to this extent in plain agar medium, but when the branched germ-tube along with the secondary conidia was transferred to a tube containing Quaker-oats medium it renewed its growth and in a faw days' time produced a white fluffy felt-like mycelium. mycelium continued its growth and after about three weeks produced many white, compact, almost round, sclerotial bodies. The sclerotia later on turned orange-yellow and finally olivegreen in colour and became slightly powdery in appearance. On examination they were found to correspond closely to those obtained from the host plant, in having a central white portion of compact hyphae bounded by an orange-yellow zone and finally surrounded by powdery olive-green layer consisting of mature spores. The spores obtained from these bodies germinated in the usual way and produced sclerotia.

When a suspension of secondary conidia in plain agar was plated, they were found to germinate in very much the same way as the parent They became slightly swollen at first, then produced branched and septate germ-tubes bearing clusters of small pear-shaped hyaline conidia at the tips. These tertiary conidia were slightly smaller and their germ hyphae narrower and sparsely septate when compared with those obtained from the parent spores. When the germ hyphae along with the tertiary conidia were transferred to tubes containing Quaker-oats medium the usual sclerotial bodies were obtained.

Sclerotia were obtained repeatedly in the laboratory in cultures made during the months November to February. There was no sclerotial formation in cultures made during March to August and the growth of mycelium was also not typical, being mainly flat and devoid of any

aerial hyphae.

Literature dealing with the study of this fungus Ustilaginoidea virens (Cke.) Tak. is very scanty and it appears that so far only one previous investigator, Brefeld [1895], had succeeded in obtaining sclerotia and ripe spores in cultures. Fulton [1908] and Butler [1913], in spite of their repeated attempts, failed to get sclerotial masses in culture. This failure may possibly have been due to their incubating the cultures at comparatively high temperatures.

# Growth in relation to temperature

Petri-dishes containing Quaker-oats medium were inoculated with small pieces of hyphae according to the technique followed by Seth [1934] and incubated at different temperatures. Four dishes were used for each temperature. The radial spread of the fungus was measured periodically. The final measurements taken after 29 days of growth are given in Table I.

It will seen from Table I that the fungus fails to show any growth at 34°C. The optimum temperature appears to be in the vicinity of 26°C. When the plates incubated at 34°C. and 37°C. were transferred to lower temperatures, no growth took place, indicating that the fungus had been killed by prolonged exposure to these tempera-

tures.

# Longevity of the spores

Sclerotial bodies of the fungus from Ngasein type of paddy were collected at Hmawbi during the first week of December 1936 and stored at Mandalay in glass tubes at laboratory temperature. The spores from these were periodically examined, by growing them on plain agar medium to find out how long they could remain viable. The results obtained are given in Table II.

It will be noted that the spores can remain viable up to a period of only about 8 months from the time of their formation.

# Germination of sclerotia

(i) In order to study the germination of the sclerotial bodies, four glass troughs filled with soil obtained from a paddy field at Mandalay and four filled with sand were taken and the sclerotial bodies sown in these at a depth of about one inch. Two troughs containing paddy-field soil and two containing sand were flooded with water once a

TARLE I Growth in relation to temperature

Tempera- ture °C.	Radius of the colony in mm.	Remarks
37 34 32 30 29	 16·5 19·75 23·0	No growth No growth Mycelium flat, not typical Mycelium flat, not typical Mycelium mainly flat, very slight aerial growth, not
28	23.0	typical Mycelium mainly flat, very slight aerial growth, not typical
26	25.0	Mycelium white fluffy, typical. Sclerotia and spores formed
22	21.75	Typical growth, tendency to- wards formation of sclerotia. Sclerotia and spores formed seven days later

TABLE II Longevity of the spores

Date on which cultures made	Remarks
22 Dec. 1 936 6 Jany. 1937 11 March 1937 30 June 1937 4 Aug. 1937 30 Aug. 1937 6 Sept. 1937	Spores germinated Spores germinated Spores germinated Spores germinated Spores germinated Spores failed to germinate Spores failed to germinate

week to keep the soil and sand thoroughly wet. The remaining troughs were watered whenever the contents in them had dried. Sclerotial bodies were sown on 3 January 1936. Six months later each sclerotial body was found to have been reduced to a dark powdery mass consisting of spores only and there was no indication of the production of any ascus-bearing structure. These spores failed to germinate in nutrient media in spite of repeated attempts.

(ii) In another method, small pieces of the central white compact tissues of the sclerotial bodies were transferred to Quaker-oats medium. The

procedure adopted was as follows:

Sclerotial bodies were dipped in a 0.01 per cent mercuric chloride solution containing a small quantity of ethyl alcohol for one minute and then repeatedly washed with sterile distilled water. These were then placed in a glass dish containing sterile water and cut in the form of small discs by means of a sterilized scalpel. Central tissues

from these discs were then cut out and transferred to tubes containing Quaker-oats medium and incubated at the laboratory temperature.

Growth commenced on the 3rd day and in another three weeks' time, many typical sclerotial bodies developed. No ascus-bearing structures appeared though the cultures were kept in the laboratory for a period of over three months

Inoculation experiment

In order to find out if the disease could be reproduced under field conditions, the inoculation of flowers just at the time of opening and of the grains in the milk stage was carried out at the Farm. The method Mandalay consisted of inserting the spores within the glumes by means of a sterilized needle or spraying with a suspension of spores in sterilized water. The spores of the preceding year, fresh spores as soon as they could be obtained and spores obtained in cultures were used. Some of the flowers after treatment were enclosed in tubes and some left exposed. The total number of flowers and grains thus treated was 2109. The inoculations were carried out during the third week of November, 1936. At the time of the examination in January, 1937, it was found that the majority of the inoculated seed had matured quite normally and all were free from infection.

Another experiment was carried out in pots. Seed of Ngasein paddy (C 24-71 type) which is known to be very susceptible to the disease, was obtained from Hmawbi. The seed was sown on 4 September 1937, after receiving the following treatments:

(a) Seed dusted with fresh spores obtained from Hmawbi Farm.

(b) Seed dusted with spores obtained in cultures.

(c) Untreated seed.

On 4 January 1938, when the final examination was carried out, it was found that all plants had matured normally and all remained free from infection.

#### SUMMARY

1. The fungus attacks individual paddy grains and transforms them into large green round masses which may be about twice the diameter of normal grains.

2. The spores on germination in cultures give rise to sclerotial bodies closely resembling those

found under natural conditions.

3. The optimum temperature for the growth of the fungus is in the vicinity of 26°C. Prolonged exposures at temperatures above 34°C. kill the fungus.

4. The spores can remain viable up to a period

of only about eight months.

5. Sclerotial bodies failed to produce any ascus-

bearing structures.

6. Inoculation of flowers and of the grains in the milk stage failed to produce infection. Seed dusted with spores produced normal and diseasefree plants.

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# SUGARCANE VARIETAL TRIALS IN THE DECCAN CANAL TRACT

# II. PERFORMANCE OF VARIETIES IN DIFFERENT PLANTINGS AND RATOONING

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In the canal tract of the Bombay Deccan, it is customary to plant cane at different times of the year. Generally there are three well defined periods of planting, viz. (1) Adsali or post seasonal planting starting in June, (2) Pre-seasonal starting in October, and (3) Suru starting in January. The main re son for the introduction of the first two types of plantings is to secure higher cane tonnages by lengthening the period of growth. Further as they come to maturity and harvest earlier than the January planting, culti-

vators are able to secure higher prices for their gul and the factories to extend the duration of the milling season by starting the working of the factory early. It was, therefore, considered essential to conduct trials of some of the improved varieties selected previously [Vagholkar and Patwardhan, 1940] at these plantings in order to find out their suitability. Simultaneously, the ratooning power of these varieties as well as the best planting suitable for ratooning was also tested by ratooning these plantings.

#### MATERIAL AND METHOD

Five promising varieties—Co 360, 407, 408, 413 and 419 were grown in these plantings of July, October and January in a split plot design of layout with plantings as main plots and varieties as sub-plots. The replications were three and the experiment was conducted for a period of three seasons, plantings being done during the first week of the month in each case.

The basal manuring was common to all the plantings and consisted of 30,000 lb. of compost prepared mainly from sugarcane trash and 100 lb. of P<sub>2</sub>O<sub>5</sub> in the form of superphosphate. As regards nitrogenous topdressings, July planting received 300 lb. N in the proportion of one of sulphate of ammonia to two of ground-nut cake while other plantings received 225 lb. N in equal proportions of these ingredients. Harvesting was done when the maximum brix and purity were

attained in each planting, which took about 17 to  $17\frac{1}{2}$  months for July planting, 14 to  $14\frac{1}{2}$  months for October planting and 13 to  $13\frac{1}{2}$  months for January planting from the date of planting. All these plantings were ratooned after the harvest and the ratoon crop received a common dose of topdressing of 225 lb. N in equal proportion of sulphate of ammonia and ground-nut cake.

#### PRESENTATION OF DATA

Combined plantings

(1) Germination. The final germination counts which are taken at the end of six weeks in the July and October plantings and at eight weeks in January planting are given in Table I. The seed rate in all these plantings was 10,000 three-budded setts per acre. The figures are given as average of three seasons as the trend from year to year has been practically similar.

Table I
Final germination counts
(Area one cent)
(Average of 3 seasons)

		Mean				
Time for planting	Co 360	Co 407	Co 408	Co 413	Co 419	for planting**
July October January Mean for varieties †	173* 160 179 171	214 197 261 224	203 146 221 190	243 -213 241 232	218 202 238 219	210 184 228

\*C.D. for any two treatments 10·2 \*\*C.D. for means for planting 7·89 † C.D. for means for varieties 5·93

Plantings are quite significant, the order being January, July and October, the last showing the least germination in the case of all the varieties. This is surprising as both from the standpoint of temperatures and the nature of the planting material both the latter plantings have been in a favourable position. The planting material is obtained from a crop 12 months old in the case of each planting and as such it would consist of more immature canes in the case of July and October plantings. So also the minimum temperatures throughout the period of germination in both these plantings remain higher than 50°F. and as such are in fact favourable to rapid germination [Rege and Wagle, 1939]. The fall in total germination in both these plantings seems to be, therefore, due to the soil conditions as affected by rains. In the case of July plantings rains which are received often during the period of germination would keep the soil continuously moist and this is known to adversely affect the

germination. In October planting it is difficult to secure good tilth owing to the obstruction to proper cultivation operations as a result of heavy rains in September. Both these adverse factors are not experienced in the J nuary planting. These comparative studies in plantings have thus enabled one to get a proper estimate of the influence of soil on germination.

As regards the varieties, Co 413 has given the best germination and Co 360 the worst, the other varieties being intermediate, Co 408 seems to be more susceptible to the soil conditions than others.

(2) Tillering and borer counts. During the tillering phase periodical records have been kept of the plant population and the borer affected plants; but for the limitations of space only the figures at the time of earthing, which represent the maximum number of living plants, are given in Table II with the figures of total percentage of borer damage during this period. In addition the figures are given of the canes at harvest and their

percentage success on the maximum population. All the data are the average of three seasons as although some seasonal fluctuations are evident the trend from season to season has been practically similar both in the case of the varieties and

plantings.

The shoots at earthing represent the maximum tillering capacity in the varieties which is the highest in Co 413 and the lowest in Co 360 of all the varieties under experimentation. Among the plantings, these shoots are low in July planting as compared to other plantings which is mainly due to the heavy damage of borer in this planting, of which all the figures are exclusive. In spite of this smaller number of shoots at earthing, the July planting has given the highest number of canes at harvest (Table III) which is contrary to expectations as after the operation of earthing the damage of stem borer is found to be rare in

all the plantings. There is still, however, a mortality of shoots after earthing which is greater in the October and January plantings as is evident from the figures of percentage success both at two months after earthing and at harvest. The former figures of percentage success show that this mortality is mainly due to the smothering of small shoots by the operation of earthing and it is evident that such small shoots are more in the October and January plantings than in the July one. As regards the borer damage there is nothing much to choose between the varieties. Only Co 419 and Co 407 have given some indications of lower susceptibility than others. It would, however, be possible to reduce its depredations to a very great extent by adjusting the plantings.

(3) Harvest data. These are given in Table III as average of three seasons. There is a significant. fall in cane tonnages from July to January plantings.

Table II

Developmental data

(The figures of counts are reduced to one cent area)

(Average of three seasons)

	Co 360	Co 407	Co 408	Co 413	Co 419	Mean for planting
July planting (1) Shoots before earthing (2) Per cent success—after 2 months—on (1) (3) Per cent success at harvest or (1)	517 90·1 76·6	749 85·4 66·5	797 80·2 60·3	1141 79·4 43·5	656 83 · 7 68 · 3	772 83 · 8 63 · 0
(4) Per cent borer attack	20.2	10.1	20.5	16-1	12.8	15.9
October planting						
(1) Shoots before earthing	569	934	873	1385	875	927
(2) Per cent success—after 2 months—or. (1).	72.4	47.5	49.7	34.1	53.4	51.4
(3) Per cent success at harvest on (1)	68.50	44.83	46.34	31.67	50.62	48.3
(4) Per cert borer attack	2.1	1.4	1.3	1.3	1.0	1.4
January planting						
(1) Shoots before earthing	465	970	847	1184	849	863
(2) Per cent success—after 2 months—on (1).	73.1	42.5	49.0	43.1	53.5	52.2
(3) Per cent success at harvest on (1)	61.31	36.91	43.23	32.99	47.33	44.35
(4) Per cent borer attack	7.0	5.2	5.9	4.4	6.5	5.8
Mean for varieties						
(1) Shoots before earthing	517	884	839	1237	793	854
(2) Per cent success—after 2 months—on (1).	78.5	58.5	53.0	52.2	63.5	62.5
(3) Per cent success at harvest on (1)	68.8	49.41	49.96	36.05	55.42	51.88
(4) Per cent borer attack	9.8	5.6	9.2	7 · 3	6.8	7.7

So far as the October and January plantings are concerned, the higher tonnages in the former are entirely due to the longer period it gets for development as nitrogenous topdressings were the same in both these plantings. Flowering is definitely seasonal invariably occurring some time during the months of October and November according to the inherent characteristics of the varieties. It has been thus observed that all these

three plantings practically flower at the same time according to the characteristics of the varieties and as a result the earlier plantings of July and October get a longer period for development than the January one. In the case of July planting, the increase in yields over the other plantings cannot, however, be entirely attributed to the longer period of growth as there has been an increase in nitrogenous topdressings by 75 lb. N,

the dose being 300 lb. N as against 225 lb. in other

plantings.

Although the number of canes at harvest has shown similar gradient as in cane tonnages in these plantings, the increase in cane tonnages in July planting cannot be entirely ascribed to the higher number of canes, as weight per cane is also definitely higher. In the case of the other two plantings the higher tonnages in the October planting is mainly due to larger number of canes. In fact the first three varieties in this planting are showing a slight reduction in weight per cane than that in January planting. This low figure for weight per cane in this planting in spite of longer period available for growth clearly indicates

that the system of manuring in both these plantings with equal quantities of nitrogen is not a correct one and it would be necessary to give a higher quantity of nitrogen in the October planting than in the January one. Higher doses of nitrogen however leads to lodging of the crop specially in early plantings. In the case of July planting, for instance, lodging is observed from May onwards when the wind velocity increases and in one such experiment with 1000 lb. N more than 50 per cent of the crop was damaged due to driage, rat attack, etc., after lodging in the case of Co 419 eventually encouraging production of water shoots. In general it is estimated that in the canal tract as a whole, the loss in the tonnages in

Table III

Harvest data

(Average of three seasons)

마음 사람들이 있는 사람들이 하는데 있다. 중요 중에 시를 보고 있는 것 같아 있다고 있다.	Co 360	Co 407	Co 408	Co 413	Co 419	Mean for planting
			•			
July planting	49.9	49.8	60.3	58.6	66.4	57.0
Weight of canes in tons per acre .	39,600	49,800	48,100	49,900	44,800	46,400
No of canes per acre	3.00	2.55	3.18	3.32	4.05	3.22
Weight per cane in lb.		19.51	19.70	19.27	20.59	19.64
() Briv at $17.5^{\circ}$ C.	19.12	17.08	17.38	17.69	17.60	17 - 27
	. 16.58		13.36	12.70	13.01	13.27
2) Fibro per cel t	. 12.41	$\begin{array}{c} 14\cdot88 \\ 11\cdot5 \end{array}$	12.3	11.5	12:0	11.8
7) (' (' S Der Cont	11.5	5.69	$\frac{12.3}{7 \cdot 21}$	$\frac{1}{6} \cdot 72$	7.97	6 · 65
8) C. C. S. in tons per acre.	. 5.65		862	781	984	770
3) C. C. S. in tons per acre.  9) Net profit Rupees per acre	. 605	615	302	101	14.33	
October planting		40.6	49.3	51.4	54.9	47.6
1) Weight of canes in tons per acre	$42 \cdot 0$		40,500	43,900	44,300	41,900
o) No of canes per acre	39,000	41,900	2.11	2.75	3.00	2.5
3) Weight per cane in lb.	$2 \cdot 52$	2.31	$2\overline{1} \cdot 10$	20.50	21.20	21.1
4) Prize at 17.5°C.	. 20.38	22.69	18.84	18.99	18-87	19.0
5) Sucrose per cent in juice	. 18.03	20.39	15.36	13.34	12.64	14.2
a) Tilling nor cont	. 13.65	16.43		13.6	13.3	13.2
TV CI CI S ner cent	. 12.5	13.7	$\begin{array}{c} 12\cdot 8 \\ 6\cdot 27 \end{array}$	6.96	7.29	6.2
of C S in tons nor acre.	. 5.25	5.54		869	921	75
9) Not profit Rupees per acre	. 592	640	756	600	77	
January planting		00.7	42.8	46 · 7	48.5	42.6
v. vit	. 36.0	39.1	36,600	39,100	40,200	36,00
No of cames per acre	28,500	35,800	2.55	2.59	2.82	2.6
2) No. of canes per acre 3) Weight per cane in lb.	2 · 82	2.36		$21 \cdot 02$	20.65	21.6
1) Bris at 17.5°C.	. 20.83	22.77	21.77	18.41	18.96	18.8
E) Garange ner cent in dilice		$20 \cdot 10$	18.80	14.55	12.76	14.4
6) Fibre per cent 7) C. C. S. per cent	. 11.98	15.95	16.92	12.7	$13 \cdot 2$	12:9
TO C S per cent	. 12.7	13.4	12.5	5.86	6.39	5.5
old C S in tong per acre.		$5 \cdot 24$	5.33	715	802	65
9) Net profit in Rupees per acre .	. 528	617	639	119	302	
Mean for varieties			F0.0	52.2	56.6	49.1
(1) Weight of canes in tons per acre	. 42.6	43 2	50.8	44.300	43,100	41,40
9) No of canes per acre	. 35,700	42,500	41,700	2.89	3.29	2.8
21 Weight per cane in lb.	2.78	2.41	2.61	20.1	21.1	20.7
(a) Brivet 17.5°C.	20.1	21.7	20.6	18.36	18.48	18.3
Ex Corongo ner cent in lince	. 17.59	19.19	18.34	13.53	12.80	13.9
6) Fibre per cent	. 12.68	15.75	15.21	12.6	12.8	12
(7) C. C. S. per cent	. 12.2	12.9	12.5		7.22	6
ioi C C S in tons per acre	. 5.2	5.49	6.27	6.51	902	75
(9) Net profit in Rupees per acre	580	624	749	788	902	

Table IV

Periodical brix and purity readings in the combined plantings
(Average of three years)

	w lay way or	Mid-C	October	Mid-No	ovember	Mid-D	ecember	Mid-Ja	anuary
Planting		Brix at 17.5°C.	Purity per cent	Brix at 17.5°C.	Purity per cent	Brix at 17.5°C.	Purity per cent	Brix at 17.5°C.	Purity per cent
						-			
Co 360						. • 1			
July		18.5	87.6	18.3	87.1	18.3	86.8		
October	- 1	$19 \cdot 2$	88.0	19.5	88.9	20.5	90 1		
January .				16.9	81.8	18.7	86 · 2	19.9	88.8
Co 407								- 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	
July		$20 \cdot 2$	$90 \cdot 2$	19.3	88.3	19.8	89.4		
October .		19.1	86.9	20.8	89.3	21.5	88.6		
January .	.		••	17.7	80.5	20.6	85.6	22 · 1	87.7
Co 408									
July		17.7	86.2	18.8	39.4	19.1	89.8	• •	
October .	.	17.3	85.4	18.8	88.5	20.4	89 · 2	•	89.4
January .	•		••	17.1	84.1	19.3	88.0	20.7	89.4
Co 413									
July		18.3	88.5	18.6	88.9	19.4	90.1	• •	
October .	· · ·	16.9	85.8	18.7	88.9	20.4	90.2		
January .	$\cdot$ $\mid$			16.9	83.3	18.9	27.6	20 · 2	89 1
Co 419									
July		19.0	89.0	19.6	89.4	20.0	87.7		
October .		16.4	81.4	17.7	84.0	20.5	89 · 2		
January .			1	15.9	78.1	19.0	84.8	20.6	86.3

Table V

Ration-developmental data
(The figures of counts are reduced to one cent area)
(Average of three seasons)

1	Co 360 2	Co 407	Co 408 4	Co 413 5	Co 419 6	Mean for planting 7
July planting	505	727		1,180	0.00	coo
(1) Shoots at start	367	725	559	1176	- 667	699
(2) Shoots before earthing	488	741	672	1085	693	736
(3) Per cent success on before earthing count	63.7	51.2	56.7	43.0	54.0	53.7
(4) Per cent borer attack October planting	9.5	6.8	6.9	$5\cdot 4$	6.7	7-1
(I) Shoots at start	327	556	411	991	508	559
2) Shoots before earthing	429	531	581	759	551	570
(3) Per cent success on before earthing count	70.1	66.0	62 · 1	56.3	$64 \cdot 9$	63.9
4) Per cent borer attack	9.5	6.8	6.6	4.5	6.3	6.7
January planting .						
1) Shoots at start	311	547	469	945	655	585
2) Shoots before earthing	393	559	566	961	644	625
3) Per cent success on before earthing count	69.4	57.7	64.9	40.7	54.9	57.5
4) Per cent borer attack	10•2	7.4	6.8	5 · 1	6.1	7-1
Mean for varieties						
1) Shoots at start	335	609	480	1037	. 610	614
2) Shoots before earthing	437	610	606	935	629	644
3) Per cent success on before earthing count	67.7	58.3	61.2	46.7	57.9	58.4
(4) Per cent borer attack	9.7	7.0	6.8	5.0	6.4	7.0

TABLE VI

Ratoon-harvest data
(Average of three seasons)

	Co 360	Co 407	Co 408	,Co 413	Co 419	Mean fo planting
	2	3	4	5	6	7
July planting	All the second s					
nuy panung				Property of	2.1	
(I) Weight of canes in tons per acre .	29.35	29.04	30.24	38.07	37.63	32.87
(2) No. of canes per acre	31,100	37,100	38,100	36,700	37,400	38,200
(3) C. C. S. in tons per acre	4.26	4.16	3.98	5 · 29	5.43	$4 \cdot 62$
(4) Brix at 17.5°C	$22 \cdot 77$	23.17	21.75	22.03	23.02	$22 \cdot 55$
(5) Sucrose per cent in juice	20.63	20.97	19.46	20.01	20.54	$20 \cdot 32$
(6) Fibre per cent in cane	13.41	16.35	15.31	14.82	13.68	14.71
(7) Sucrose per cent in cane	. 16.55	16.04	15.04	16.17	17.36	$16 \cdot 23$
(8) C. C. sugar per cent	14.53	14.23	13 · 24	13.83	14 · 27	14-02
October planting						
(1) Weight of canes in tons per acre	30 · 17	29.14	27.60	39.80	34.64	$32 \cdot 27$
(2) No. of cames per acre	30,100	35,000	36,100	42,700	35,800	35,900
(3) C. C. S. in tons per acre.	4.34	3.75	3.48	5.42	5.07	4.41
(4) Brix at 17.5°C.	22.98	$21 \cdot 97$	20.88	21.72	23 · 01	22.11
(5) Sucrose per cent in juice	20.57	19.35	18.56	19.65	20.75	19.77
(6) Fibre per cent in cane	13.52	17.07	15.55	14.88	13.61	15.32
(7) Sucrose per cent in cane	16.92	15.99	15.31	16.19	$17 \cdot 23$	16.33
(8) C. C. sugar per cent	14.35	12.72	12.53	13.56	14.59	13.55
January planting						
(1) Weight of canes in tons per acre .	30.04	27.61	26.65	39.21	34.08	31.51
(2) No. of canes per acre	27,300	22,300	36,700	39,100	35,300	32,100
(3) C. C. S. in tons per acre	4.39	3.85	3.50	5.21	4.94	4.38
(4) Brix at 17.5°C.	22.78	23 · 14	21.64	21.41	22 87	22.37
(5) Sucrose per cent in juice	20.75	20.83	19.41	19.46	20.57	20.20
(6) Fibre per cent in cane .	. 13.75	17.19	15.88	15.59	14.27	15.33
(7) Sucrose per cent in cane	. 16.80	16.93	16.11	16.08	17.13	16.71
(8) C. C. sugar per cent	. 14.60	13.86	13.11	13.32	14.38	13.85
Mean for varieties						
(1) Weight of canes in tons per acre	. 29.85	28.60	28 · 11	39.08	34.5	32 · 22
2) No. of canes per acre	. 29,500	31,500	37,000	39,500	36,200	35,400
3) C. C. S. in tons per acre · . ·	. 4.33	3.92	23.65	5.31	5-15	4.47
4) Brix at 17.5°C.	. 22.84	$22 \cdot 76$	11.42	21.72	22-97	22.34
5) Sucrose per cent in juice	. 20.65	20.05	19.14	19.71	20.62	20.10
6) Fibre per cent in cane	. 13.56	16.87	5.58	15.03	13-85	15 · 12
7) Sucrose per cent in cane	. 16.76	16.32	15.49	16.15	17.24	16.42
8) C. C. sugar per cent	14.49	13.60	12.76	13.57	14-41	13.81

the July planted crop of Co 419 would be between 20 to 25 per cent while in the case of POJ 2878 it is much less. In the case of the experiment under discussion in which the nitrogenous topdressing was much lower than what is the common dose in this tract, the crop shows lodging

Cane tonnages at tonnages C.D. for individual treatments 4 27 3040 C.D. for mean for planting 3 23 4157 C.D. for mean for varieties 2 71 1736

with the heavy rains of September only and even then this lodging is not very severe except in the case of Co 360. The actual damage to tonnage by lodging is found to vary between 5 to 10 per cent.

The lodging has further the deleterious effect on the quality of juice. In all these plantings harvesting is done when the cane shows very little further increase in brix and purity. The brix as well as the sucrose content is however much less in July planting than in others. The percentage C.C.S. which is a calculated figure according to Srivastava's formula shows a fall of more than 1 per cent in this planting, but owing to high cane tonnages the C.C.S. per acre comes to be higher than in the other plantings. This increase in

C.C.S. is however insignificant as compared to that in October planting although in cane tonnage the July planting is quite significant. It is interesting to note that for an increase of 9.4 tons of cane per acre in July planting, the increase in C.C.S. per acre comes out to be only 0.39 tons. In the case of October and January plantings the cane shows similar figures of brix and purity at the time of the attainment of maximum maturity which comes up at a later stage in the January planting. It is only in the cane tonnages that the former planting surpasses the other and gives therefore higher sugar per acre than the latter. Among the varieties Co 360 which possesses a greater tendency to lodging, has shown lower sucrose content than others even in other plantings owing to lodging of some canes. The percentage fibre is low in the July planting as compared to

Exactly similar trend is also observed in the net profit per acre (Table III). These figures of net profit are calculated on the pre-war figures of expenditure and the price of sugar. It would be seen that on the whole there is very little difference between July and October planting in this respect. Only the January planting shows a distinctly lower figure than the other two plantings. Periodical brix and purity figures at early stages from October onwards have shown that all the varieties do not show early attainment of maturity in July planting over October planting. On the other hand, Co 360, because of its heavy lodging in July planting, has actually shown inferior performance. Co 419 is the only variety which has shown distinctly early maturity in July planting throughout the period. The data for average of the three seasons are given in Table IV.

## Ratoon of plantings

The ratoons were kept after the harvest of these plantings by the middle of December in July planting closely followed by that of October planting. In the case of January planting, the harvesting was done in the last week of January. There was thus a delay in ratooning by about a month in the case of last planting. A dose of 225 lb. N in equal proportion of sulphate of ammonia and cake was given in three topdressings by the usual method. The developmental data are given in Table V and the harvest data in Table VI as average of the three seasons. It would be seen that on the whole there is not much difference in the rationing performance in these plantings and ratoons of the plant crop harvested by the end of January has come out as successful as that of other plantings harvested in December. It has been, however, observed from other experimental work that ratoons of later harvest are adversely affected specially due to the borer attack. The number of shoots both at start as well as at earthing up is much higher in the case of July planting than in others; but this difference is not so much reflected in the canes at harvest. In addition the average weight per cane is slightly less in this planting resulting in the production of almost the same cane tonnages in the case of all the three plantings. As regards the quality of cane, there is some indication of the better per-The formance in the ratoon of July planting. percentage fibre is low, which has shown a higher percentage of C.C.S. in this planting than in others. The differences are not of sufficient magnitude to be significant. Among the varieties, Co 360 seems to be the worst ratooner and Co 413 the best from the standpoint of the production of shoots. There is also an indication of greater borer attack in Co 360 than in others. the standpoint of tonnage, however, Co 360 equals Co 407 and Co 408 owing to greater weight per cane. Co 413 and Co 419 have given significantly higher tonnages than these varieties and among these even Co 413 seems to be superior to Co 419.

#### SUMAMRY AND CONCLUSIONS

In the Canal Tract of the Bombay Deccan, three distinct plantings are generally adopted in the case of sugarcane and investigations described in these pages deal with their relative importance as well as the suitability of the varieties for the plantings. Ratooning power of these plantings has also been studied. The conclusions are summarized below :

(1) There is a possibility of obtaining very low germination in the October planting due to the difficulty of conducting proper cultivation operations as a result of heavy rains in September which is a common feature in this tract. From the standpoint of germination January planting has come out the best. Among the varieties, Co 413 has shown a very good germinative power and Co 360 the least.

(2) July planting is characterized by a severe borer attack which reduces the number of shoots at earthing. In the case of other plantings although the number of shoots at earthing up remain much higher, a large mortality is caused during the operation of earthing by smothering of tillers, which seem to be more in these plantings than in the July one. As a result at harvest July planting has given a greater number of canes than others. Among the varieties, profuse tillering is observed in Co 413 which, however, is found to be not of much use from the standpoint of increasing the number of canes at harvest time.

(3) There is a significant increase in cane tonnages from January to July planting. In the

case of July planting it is due to both higher number of canes and weight per cane while in the case of October planting it is entirely due to increase in the number of canes. Owing to the poorer quality of juice, however, the C.C.S. per acre, is practically similar in both these plantings. There is also practically no difference in the net profit per acre in both these plantings. The only advantage of the July planting has been the attainment of early maturity in certain varieties as Co 419 which helps the sugar factories to start the crushing season earlier, and the cultivators to secure higher price for their gul. The January planting has shown a poorer performance in all

respects. Among the varieties, Co 408 and Co 419 are found to be more suitable for July planting than others.

(4) The rations of all these plantings have shown practically similar performance at harvest. Among the varieties Co 360 seems to be the worst rationer and Co 413 the best.

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# RESEARCH NOTE

# A PRELIMINARY NOTE ON THE QUICK SEDIMENTATION OF LIME JUICE FOR THE MANUFACTURE OF LIME JUICE CORDIAL

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(Received for publication on 31 August 1944)

LIME juice cordial, an important citrus fruit beverage is prepared from the clarified lime juice, i.e. juice from which all pulp, seeds, etc., have been eliminated. The method usually practised in this country by the manufacturers of lime juice cordial, for the clarification of lime juice consists of storing the juice (after addition of the preservative) in tall wooden vats and syphoning off the juice after sedimentation. This method takes two to four months (and even more) before the juice is clear enough for final filtration through filter presses for the preparation of the cordial. This necessitates a large number of vats and a huge storage space and consequently a good deal of capital of the manufacturers is locked up. In addition, the supply of the prepared cordial is often interrupted and delayed due to slow clarification process. A need has, therefore, been felt by the trade for sometime past, for a method which would quickly clarify the juice. The authors have studied this problem under the Special Fruit and Vegetable Preservation Scheme, Lyallpur, financed by the Imperial Council of Agricultural Research.

The following methods were tried for the clarification of the juice on a laboratory scale:

1. Passing the juice through Sharples' Super Centrifuge.

2. Filtration through (a) sand and charcoal filters, (b) Sietz filtering apparatus.

3. Treating the juice with (a) 5·0 per cent Fuller's Earth, (b) 6·0 per cent activated carbon, (c) Sand, (d) 5·0 per cent kaolin treated with tartaric acid, and (e) 0·1 to 5·0 per cent ordinary kaolin after ignition.

GIPD-3-2 IC of AR-7-8-45-650

4. Juice treated as under (3) was filtered through Sietz apparatus and under suction in a Buchner Flask.

5. Treatment of juice with tannin and gelatin in different proportions (0.0085 per cent tannin in combination with 0.001126 to 0.03716 per cent

gelatin).

Out of all the above treatments, the tanningelatin method gave the most encouraging results. Complete clarification of freshly extracted juice was obtained in this case in four to six days. Other treatments either did not yield a fair degree of clarity or were rather too slow in their action. No foreign undesirable taste was imparted to the juice clarified by the tannin-gelatin method.

The applicability of this method to old (stored) juice has yet to be tested. It is also proposed to give the method commercial trial in some factories this summer. Experiments are already under way, and the detailed results will be published as soon as commercial trials have been completed.

Note.—It is gratifying to note that the tannin-gelatin method developed by the authors, is now being successfully used in Mr Mitchell's squash and cordial factory at Renala Khurd (Punjab) for quick clarification of lime juice for cordial making. The remarks made by this firm about this method are reproduced below:

"We are greatly indebted to you for giving us this new method which has proved of immense use to us. As we have already pointed out our cordial manufacture would have been seriously delayed, if we had not got this method in time. Until now we have to keep large quantities of lime juice in stock and even when extracting new juice we keep about four months' supply of old lime juice as the new juice is supposed to take at least four months to clear up, but with this method, we hope to be able to reduce our stocks and make use of the supplies of fresh limes which can be had practically all the year round."

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# ORIGINAL ARTICLES

# STUDIES ON THE METHODS OF ESTIMATING TOTAL BACTERIAL COUNTS IN THE SOIL

By W. V. B. Sundara Rao, S. V. Desai and M. K. Reddy, Imperial Agricultural Research Institute, New Delhi

(Received for publication on 27 June 1944)

From a study of the micro-organisms in pure cultures, the morphology and general physiology of the organisms and an indication of the limits of their activity can be obtained. But to obtain information regarding the way in which the micro-organisms of the soil live and work under natural conditions, we have to take recourse to studies in soil. Of the several methods of studies in soil, the statistical method in which the number of bacteria in the soil are estimated and are studied in relation to other quantities (factors) under strict statistical control deserves a trial. For this, among other data, an exact number of the total number of micro-organisms in the soil is required.

Plate method and direct count method are the usual methods employed for estimating total bacterial numbers. Earlier studies with the plate count method of Thornton [1922] showed that with 10 plates the per cent standard error between plates was below 8 in eight out of ten cases while with 3 plates the value was over 11 in nine out of thirteen cases (Table I). Thus a minimum of ten plates appears to be necessary to obtain an error within 10 per cent. However, the bacterial numbers obtained by the plate method are not likely to represent the total bacterial content of the soil in view of the following generally recognized limitations:

(1) The strict anaerobic micro-organisms are excluded as well as the important groups of nitrifying, non-symbiotic nitrogen fixing, sulphur oxidizing bacteria, to some extent denitrifying, symbiotic nitrogen fixing, pectin and cellulose decomposing bacteria. (2) High dilutions are necessarily used so that if groups of micro-organisms are determined on the plate, those organisms occurring in small numbers will give a rather inaccurate count.

After a trial of over 12 years Thornton and Gray [1934] expressed their opinion that it was very uncertain whether those organisms that grow on the medium employed are of main importance in carrying out biochemical processes in the soil and that it was necessary to develop

the direct count method by which alone it would be possible to obtain an estimate of the total bacteria present in the soil.

Table I

Plate method

(Samples from different plots)

Sample	Mean Count.	S.E. between Plates	Per cent S.E.	c.v.
Using 10 plat	es.	Delhi Soil.		
1	80	±4·1	±5·13 [	16.22
2	70	±6.96	±9·5	30.11
3	82.2	±5·9	±7.2	22.77
4.	80.3	±3.6	±4·41	13.95
5	132	±10·9	±8.3	26.25
6	115	±6·0	±5·2	16-44
7	85	±6.6	±7·1	22-44
8	98	±9.7	±10.9	34 47
		Pusa Soil.		
1	66	±8.6	±5·5	1.7.39
2	64	±0.66	±1·0	3-16
Jsing 3 plate	<b>S.</b>	Guntur Soil.		
1	87.6	2.33	2.7	4-18
2	93.6	4.3	4.8	7.95
3	60.0	2.6	4.3	7.45
	Pusa	Soil (root zone)		
1	35.3	5.8	16.0	27.71
2	29.3	5.0	17.1	29.62
3	34.7	5.8	16.7	28.95
4	45.3	6.7	15.2	26.97
5	35.3	6.9	19.6	33.95
	Pusa Soil	(away from root z	one)	
1	24.7	4.8	19.4	33.61
2	20.3	4.3	21·1	36.54
8	23.7	0.67	2.8	4.85
4	, 28.0	6.4	22.2	••
(Saud Fredrick)	11.7	2.7	11.8	38.45

A suitable staining technique for enabling bacteria to be distinguished in soil films was developed by Conn [1918] and further modified by Winogradsky [1925]. Two very serious difficulties had, however, prevented this technique from being developed into an exact quantitative method. These were (a) the difficulty of estimating with any accuracy the minute mass of the soil examined in a thin film, and (b) the fact that the bacteria were not distributed at random over the film from which the microscope fields had to be taken as samples. These difficulties were eventually overcome by means of a ratio method, developed by Thornton and Gray [1934], the principle of which was as follows:

'A suspension of indigotin particles averaging one micron in diameter is made up and the number of particles per c.c. is determined by means of hæmocytometer. A known mass of soil is shaken up in a known volume of this counted suspension. Films of the resulting mixture are prepared and stained with an acid dye like Erythrosine (sodium or potassium salt of tetraiodo fluorescein) which stains only organisms but not organic matter (since most of soil organic matter is in humic acid condition). The bacteria and indigotin particles are counted in a suitable number of random microscope fields and the ratio of bacteria to indigotin is determined. Since the absolute number of indigo particles added per gram of soil is known, the number of bacterial cells is calculated from the ratio according to the formula  $O = 5Y\frac{B}{I}$ —(1)

where Y = the number of indigo particles per c.c. of the suspension, 5 c.c. of which were added per gram of soil, and B and I the total number of organisms and indigotin particles respectively, counted in the films.

The calculation here is independent of the quantity of the soil in the film. The ratio of the bacteria to indigotin show a random distribution over the films and the actual number of bacteria per gram of soil is calculated from this ratio.'

Thus the two serious difficulties that prevented the direct count method from becoming an exact quantitative method were overcome. An attempt was made to test this method in these laboratories

#### EXPERIMENTAL

The soil samples selected were (1) Pusa soil (silty loam), and (2) Guntur soil (heavy black cotton soil) differing widely in mechanical composition as below:

Soil	Sand	Clay
Pusa	70%	5.6%
Guntur	14.6%	64%

The soil sample in each case was sieved through 1 mm. sieve and was spread evenly on a large sheet of paper and divided into four different portions and one composite portion was made therefrom. Counts were taken from all the five portions. The procedure followed for the estimation of the bacterial numbers by the direct count method was the Thornton's method [1934], with some alterations in some details of technique which were found necessary.

Alterations made in the method. While Thornton [1934] employed an indigotin suspension containing 500 million particles per c.c., the indigotin suspension employed during the present investigation was made to contain only 25 to 35 millions. Indian tropical soils contain usually less number of organisms than the soils of the B (Bacteria)

temperate regions. If the value  $\frac{D}{I}$  (Indigotin) is made large by reducing the number of indigotin particles then slight errors in counting bacteria will not alter the ratio much and as such wide variations are not likely to be introduced, as a result of this error, in the final calculated number of organisms in the different aliquot (of equal quantities) of the same soil sample.

Further, an alteration had to be made in the method of counting the indigotin particles and bacteria on the soil film. Thornton and Gray [1934] suggested that it would be best to insert in the eye piece a glass disc ruled with a square having 2 mm. side and to take only the area covered by the square as the field. But in our experiments the square was not inserted. The counts were taken over the entire field. since the bacteria and indigotin particles in an area of 2 mm. square were too few. Better agree-

ment in the value  $\frac{B}{I}$  could be had since the field area was greater.

# Testing the accuracy of the method

(1) Agreement between replicate microscope fields and drops. It was mentioned in the introduction that a serious source of error in many direct methods of counting soil organisms had been the uneven distribution of stained bacteria over the films. In the method developed by Thornton and Gray [1934] it was claimed that the ratio of number of organisms to numbers of indigotin particles would be more uniformly distributed than the organisms taken by themselves. This was now tested by calculating the  $x^2$  indices of dispersion between replicate microscope fields. These values were calculated for five series of counts from three soils examined. The results are shown in Table II where column A gives the number of degrees of freedom 'n' for each count, columns B and C show  $x^2$ indices and the corresponding value of P for the counts of micro-organisms taken by themselves. Here the micro-organisms were un-

\* x² Indices of dispersion for ratios of organisms to indigo particles and for organisms alone in replicate microscopic fields

TABLE II

		A	В	c	D	E
Soil		N	x2 of organisms alone	P	x² of ratios	P
Pusa away from roots	1	63	450-21	<0.001	17.89	Nearly 1
	2	47	232.0	< 0.001	5.96	,,
	3	55	792-4	<0.001	9.53	,,
	4	63	267.73	<0.001	3.28	,,
	5	63	132-29	<0.001	6.38	,,
Pusa root zone .	1	63	445-84	<0.001	4.68	,,
	2	55	253.72	< 0.001	4.41	,,
	3	63	432-11	<0.001	8.5	,,
	4	63	466-8	< 0.001	7.18	77 gs
	5	55	516-67	<0.001	11.36	7,5
Guntur , ,	1	63	133-9	<0.001	4.99	,,
	2	63	275.51	<0.001	5.09	,,
	3	63	221.51	<0.001	5.29	
	4	63	242-13	<0.001	4.26	,,
	5	63	332-27	<0.001	10.16	**

evenly distributed over the films, the values of  $x^2$  being excessive. Columns D and E show  $x^2$  indices with their values of P for the ratios derived from the same replicate microscope fields. These values denote that there was randomized distribution of the ratios. Thus while the microorganisms were unevenly distributed over the films, the ratios (of number of micro-organisms to the number of indigotin particles from the same microscope field) did not show excessive variability over these films. This was probably due to the surface tension forces acting similarly upon the micro-organisms and indigotin particles which were nearly of the same size.

When a large mass of data is to be examined it will be convenient to take drop instead of field as unit. A comparison of replicate drops also shows any variability that may be introduced during the making of the drops. Table III shows the  $x^2$  values for the drop ratios of 5 portions of each of the soils examined. In each portion of the soil examined it will be observed from the corresponding  $x^2$  values, that the ratios were distributed at random.

Table III

Counts of micro-organisms

Sample	Organisms	Indigo	Ratios	$x^2$ between drop ratios $N = 15$	Micro-organism per gram of dry soil, in millions
1. From fi	ive portions	of a samp	ole of soil	from Pusa (a	way from
$\frac{1}{2}$	$\begin{array}{c c}  & 1125 \\  & 2065 \\  & 1532 \end{array}$	557 1038 757	2.029 1.990 2.024	1.752 0.811 1.521	97.65 96.80 97.85
3 4 5	1471 1941	722 951	2·034 2·040	2·000 1·781	98·50 98·50
					± 0.315 ± 0.322%
2. From fi	ve portions o	f a sampl	e of soil fr	om Pusa (ro	ot zone)
1 2 3 4 5	3214 2731 3392 2987 2400	797 677 844 734 596	4.033 4.034 4.019 4.070 4.028	1.008 0.496 0.362 0.542 1.1565	195.0 195.0 194.3 196.8 194.7 ±0.43
3. From fi	ve portions o	f a sampl	e of soil fr	om Guntur	±0.22%
1 2 3 4 5	2987 3606 2834 3177 3723	476 581 456 496 590	6.275 6.206 6.215 6.405 6.310	0·811 0·476 1·567 1·205 1·238	$\begin{array}{c} 1058\cdot00 \\ 1046\cdot40 \\ 1047\cdot85 \\ 1080\cdot00 \\ 1055\cdot00 \\ \pm 6\cdot02 \\ \pm 0\cdot413\% \end{array}$

(2) Consistency of results. Two workers made counts from different microscope fields from every drop on the slides prepared from each of the five portions of the Pusa soil sample. In the absence of

a personal error, their counts should agree as random samples. Table IV shows the number of micro-organisms and of indigotin particles counted and the ratios obtained by each worker from each of the five portions. The sum of  $x^2$  indices of

dispersion between the two workers' counts from individual drops of each portion were entered in column 11 of Table V. The two series of counts agreed within random sampling, personal error being very little. The calculated numbers agreed closely.

# TABLE IV

Counts of micro-organisms by two workers from five portions of a sample of soil from Pusa
Plot No. 3-A

(October sample away from roots)

# DIRECT COUNT METHOD

No.	Slide 1	Slide 2	Slide 3	Slide 4	x <sup>2</sup> for slide ratios	Total	Organisms in millions per gm. of soil	Slide 1	Slide 2	Slide 3	Slide 4	x2 for slide ratios	Total	Organisms in millions per gm. of soil
	_ <u>b</u>	$\frac{b}{1}$		$\frac{b}{1}$		<u>B</u> 1		_b1	<u>b</u>	<u>b</u> 1	<u>b</u>		$\frac{B}{1}$	
1	528 236	194 194	189 91	214 106	0.1305	1125 557	97•65	811 396	409 202	644 323	395 101	0.1369	2259 1112	97-78
2	95	426 217	762 369	$\frac{785}{401}$	0.4081	2068 1033	96-80	••	$\frac{271}{131}$	$\frac{518}{256}$	471 245	0.3445	$\frac{1260}{632}$	96-35
3	891	484 234	150 71	$\frac{307}{149}$	0.4690	1532 757	97.75	$\frac{432}{210}$	$\frac{472}{202}$	$\frac{556}{279}$	562 281	2.5630	2022 981	96-66
4	360 181	399 195	380 187	$\frac{332}{159}$	0.1265	$\frac{1471}{722}$	98-50	675 327	$\frac{874}{427}$	522 276	520 246	0.3027	2661 1276	100.83
5	588 280	451 225	444 220	458 226	0.2984	1941 951	98•50	850 412	655 314	538 256	$\frac{478}{228}$	0.0463	$\frac{2521}{1210}$	100.73
							S.E. = ± 0·315 (± 0·322%)							S.E. = ± 0·307 (± 0·312 %)

Formula for  $x^2 = \frac{1}{BI} \sum \left\{ \frac{(bI - Bi)^2}{b + i} \right\}$ 

Where b and i are the bacteria and indigo counted in each slide and B, I are the total bacteria and indigotin particles counted

# TABLE V

Counts of micro-organisms by two workers from five portions of a sample of soil from Pusa Plot No. 3-A

	(	Counts by	W. B. S. Ra	0	Cot	ints by M. I	K. Reddy					
Sample	Organisms	Indigo	Ratios	*x* between slide ratios	Organisms	Indigo	Ratios	x² between slide ratios	<b>n</b>	between workers counting	Mean ratio	Millions per gm. of air dry soil
1	2	3	4	5	6	7	8	9	10	11	12	13
A . B . C . D . E . Total •	1125 2068 1532 1471 1941 8137	551 1033 757 722 951 4020	2·0197 2·0017 2·0235 2·0374 2·0211	0·1305 0·4801 0·4690 0·1265 0·2984 1·5045	2259 1260 2022 2661 2521 10723	1112 632 981 1276 1210 5211	2·0224 1·9940 2·0610 2·0850 2·0830	0·137 0·345 2·560 0·303 0·046 3·391	15 10 13 16 16 70	1.865 0.969 1.866 1.249 1.583 7.532	2·0211 1·9979 2·0423 2·0612 2·0526	97-719 96-602 98-750 99-655 99-244
Mean				n = 15				n = 15			2:03522 S.E.=	: =0·2916 (0·2963%)

Errors in sampling due to variation in grain size. The soil selected was a sample from Guntur having 8.4 per cent coarse sand. Sterilized sand was added to it to raise the percentage of coarse sand to 20, 30 and 40 and mixed thoroughly. Thus four different types of soil differing in grain size only, were obtained. Sand was sterilized by autoclaving for two hours at 15 lb. pressure.

Original soil 8.5 per cent coarse sand
 Soil with 20 per cent coarse sand
 Soil with 30 per cent coarse sand

4. Soil with 40 per cent coarse sand

In each of these soils the sample was spread on a sheet of paper and divided into four portions. Each portion was put into a separate bottle and labelled. The total bacterial counts were estimated in each portion by the direct microscope count method. Table VI shows the results obtained.

### TABLE VI

Total bacterial number variations with addition of sterile sand

DIRECT MICROSCOPE COUNT METHOD

						acterial illions p s		
I. Guntur cent).—	Soil	—orig	ginal	(cc	arse	sand	8.5	per
Portion ,, ,, Mean	1 2 3 4					1,006.5 1,036.8 953.1 977.1	} L	1.78)
II. Guntur	Soil	(coars	e san	d 2	0 per	cent)-		
Portion ,, ,, Mean	1 2 3 4					855-4 877-4 827-6 870-7 857-8	50 35	1.99)
III. Guntur	Soil	(coar	se sa	nd :	30 pe	r cent	)	
Portion ,, ,, Mean						750-8 702-8 770-4 727-7	35 36 L	·16)
IV. Guntur	Soil	(coar	se sar	nd 4	0 per	cent)	_	
Portion " " Mean	1 2 3 4					662.9 674.1 665.6 644.3	)7 l i	0·95)

From these results it appears that the percentage standard error varied between 1 and 2. Further

it can also be seen that the decrease in bacterial numbers per gram of soil corresponded to the increase in the amount of sterilized sand added.

Table VII shows the expected values and the values observed.

TABLE VII

		s. per gram of millions	Difference
Sample	Expected	Obtained	percentage
1 2 3 4	993·43 857·86 737·80 661·74	879-1 779-8 680-5	2·4 5·3 2·6

From these results it can be seen that the method is fairly accurate. The authors are greatly indebted to Rao Bahadur Dr B. Viswanath, C.I.E., F.I.C., D.Sc., Imperial Agricultural Chemist, for his advice during the course of this work.

#### SUMMARY \*

In the present investigation it was observed that in the plate count method a minimum of ten plates was necessary to obtain an error within 10 per cent. Since no single medium is suitable for the growth of all soil bacteria belonging to different physiological groups, the bacterial numbers obtained by plate method do not represent the total bacterial content in the soil. As such the direct microscope count method of Thornton and Gray [1934] was studied in detail as to the agreement between replicate microscope fields and between drops, consistency of results and the errors in sampling due to variation in grain size of the soil. This method with a few modifications in some details of the technique was found suitable to determine the total bacterial count in the soil within an error of  $\pm 2$  per cent.

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# A STATISTICAL STUDY OF THE BOLL FORMATION IN COTTON

By D. N. Nanda and Mohammad Afzal, Cotton Research Laboratory, Lyallpur

(Received for publication on 23 October 1944)

(With one text-figure)

In the previous two papers of this series [Afzal and Iyer, 1934 and Nanda, Afzal and Panse, 1944] the growth in height and the flower production in four varieties of cotton, three American and one indigenous, were studied in detail. In the case of growth in height, the exponential equation  $H=Ae^{\rm bt}$  was found to fit the data and in the case of flowering a polynomial of the

third degree of the Log  $\frac{x}{a-x} = Y = A + BT + CT^2 + DT^3$  was found suitable.

These studies have now been carried further and the boll formation in cotton has also been studied. The experimental data were collected from the Physiology Plot of the Cotton Research Laboratory, Lyallpur.

Two hundred average plants were selected at random in each of the four varieties, namely, 4F, 289F, 289F/43 and 39 Mollisoni just before boll opening started and the number of bolls picked per plant per day was recorded in each case. The bolling season was divided into intervals of 5 days and the progressive total of the number of bolls produced per plant during each interval was plotted. It was found that the

summation curves of all varieties were of the characteristic S-shape.

The data were examined by fitting the logarithmic equation of the fifth degree in the time variable and it was found that the mean squares were in a few cases significant up to the fifth degree. The mean squares up to the third degree were practically always significant and it was, therefore, considered that the third degree equation may be considered to give the best fit. The equations for the average polynomials are given below:

As in the case of flowering, the average polynomial for each variety was calculated and the sum of squares contributed by the linear, the quadratic and the cubic components of the curves and the corresponding deviations from the average regressions were calculated. It was found that the average polynomial of the third degree was significant in all varieties except 289F as will be seen from Table I.

Table I
Sum of squares of the average regressions

		4F			289F			289F/4	3		39 Mollise	oni
Due to	D.F.	s.s.	M.S.	D.F.	s.s.	M.S.	D.F.	s.s.	M.s.	D.F.	s.s.	M.S.
Linear regression .	-	176.6	176-6**	1	147:3	147.3**	1	88-23	88-23**	1	69.84	69-84**
Quadratic regression	. 1	0.4004	0.4004**	1	0.1000	0.1000**	1	0.0412	0.0412	1	0.7043	0.7043**
Cubic regression .	. 1	0.6994	0.6994**	1	0.0427	0.0427	1	0.5622	0.5622**	1	1.5113	1.5113**
Deviation from												
Linear regression .	. 6	16.17	2.696**	6	37.49	6-248**	2	10.51	5.259**	4	22.38	5.595*
Quadratic regression	. 6	2.926	0.4878**	6	2.268	0.3781**	2	0.4945	0.2472**	4	2.039	0.5097**
Cubic regression .	. 6	0.4813	0.0802	6	2.210	0.3683**	2	0.1695	0.0847	4	0.7263	0.1815**
Residual .	. 98	2.664	0.0271	77	-1-274	0.0165	48	1.597	0.0332	60	0.5850	0.0097
Total	. 119	200.0		98	190-7		57	101.6		75	97-79	

It will be seen from Fig. 1 that the deviations in the case of flower production, the rate of boll of the yearly curves from the average ones were formation was also influenced by the seasonal usually significant, indicating thereby that as factors to a great extent.

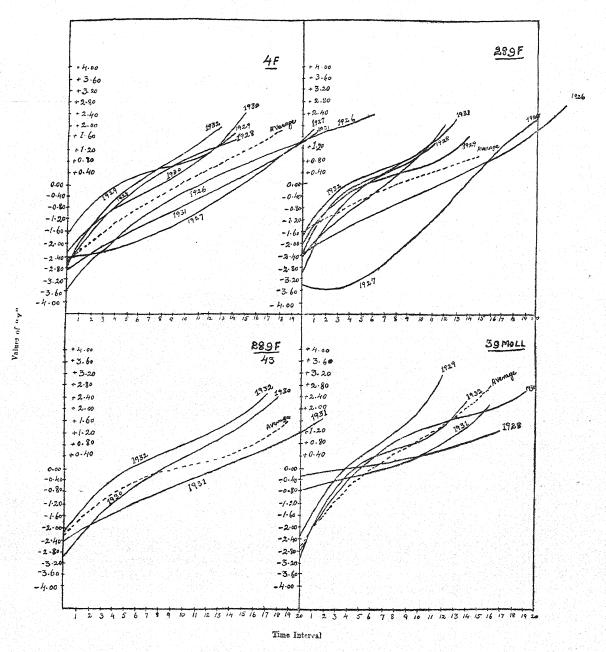


Fig. 1

Since the logarithmic equation fitted to the data was significant up to the third degree, the relative rate of boll formation cannot be considered

to be constant. It was really a function of time. The relative rates of boll formation for each variety are given in Table II.

Table II
Relative rates of boll formation

Variety	1926-27	1927-28	1928-29	1929-30	1930-31	1931-32	1932-33
4F	0·042 0·042 	0·045 0·065 	0·057 0·057 ··	0·040 0·042  0·079	0·055 0·070 0·054 0·051	0·041 0·070 0·035	0·065 0·050 0·047 0·061

It will be seen from Table II that the relative rate of boll formation was the highest in 39 Mollisoni and least in 289F/43. A reference to Table IV in the article on flower production in these varieties [Nanda, Afzal and Panse, 1944] will reveal that the relative rate of flower production was also high in the case of indigenous variety, 39 Mollisoni, and low in the exotic cottons. Amongst these latter 289F/43 had usually the least relative rates.

A comparison of the average rates of flower and boll formation is very interesting and is given in Table III.

Table III

Comparison of the average relative rates of flower
and boll production

Variety	Average rate of flower production	Average rate of boll formation	Difference between the two rates
39 Mollisoni	0-0781	0.064	0.0191
289F/43	0.0601	0.045	0.0151
4F	0.0619	0.049	0.0129
289F	0.0631	0.057	0.0061

Since bolls are formed from flowers, the rates of flowering and bolling would be the same if there were no shedding of young bolls. The difference in these two rates is, therefore, the rate of boll shedding which is shown in the last column of Table III. It will be seen from these figures that the rate of boll-shedding is highest in 39 Mollisoni. This is because in this variety flowering starts very early, towards the middle of July, when the weather is still very hot and there is very heavy shedding. 289F/43 comes nearest to Mollisoni in this character and, therefore, the rate of bollshedding is also high here. Next in order of lateness is 4F and the latest variety is 289F and the rates of boll-shedding are also of the same order in these two varieties. The very distinct fall

in the rate of boll-shedding in 289F may be due to the fact that since the flowering in this cotton starts towards the middle of September, there is very little non-dehisence of anthers [Trought, 1928] in this variety. Since 289F/43 is the earliest among the American cottons it suffers from non-dehisence for a longer period and, therefore, the rate of boll-shedding is the highest. It is, therefore, suggested that under the conditions obtaining at Lyallpur, a very early flowering variety of American cotton is not desirable and it will be interesting to note that 289F/43 does not grow well here and will probably never become a commercial variety in this part of the Punjab.

These studies have brought forth a very important point of practical utility. Here we have a means by which it may be possible to judge the adaptability of a foreign variety to the local conditions after a very short trial. If data of the type presented in Table III were collected on the foreign variety under the local conditions, the adaptability or otherwise of the new varieties could be judged, with greater confidence and with greater chances of success than is possible at present, in a very short time.

It may be mentioned in the end that the present paper is the last of a series embodying studies in growth in cotton. Heath [1932] was the first to study growth in height of cotton in South Africa while Prescott [1922] had studied flower production in Egypt. Boll formation has been studied in the Punjab for the first time. We have, thus, a complete picture of four most important varieties of cotton grown in the Punjab.

# SUMMARY

A logarithmic equation of the third degree has been fitted to the bolling data of cotton and it was found that although the mean squares were significant up to the fifth degree in a few cases, it was considered that the third degree would give a very good fit.

The relative rate of boll formation varied with the time. This had to be the case, because, as previously reported, the rate of flower production was also found to vary likewise.

It has also been possible to calculate the average rate of boll-shedding and it was found that early maturing varieties had comparatively higher rate of boll-shedding than the late maturing varieties. It was, therefore, argued that at Lyallpur a late maturing variety fits into the environmental complex much better than an early maturing one. This deduction from purely theoretical consideration has been found to be a true one as no early maturing variety can be successfully grown here.

# ACKNOWLEDGEMENT

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# COTTON JASSID (EMPOASCA DEVASTANS DIST.) IN THE PUNJAB

VI. SPECIES FOUND ON THE COTTON PLANT IN THE PUNJAB

By Manzoor Abbas and Mohammad Afzal, Cotton Research Laboratory, Lyallpur (Received for publication on 28 October 1944)

A VERY large number of species of Empoasca infest different field and garden crops in the Punjab. Some of these species have not yet been properly identified. As a very large number of insects belonging to this genus are pests of one plant or the other, it is suggested that an exhaustive. survey will be of interest not only to entomologists

but to general agriculturists also.

In the previous literature on cotton jassids, only one species, namely, *E. devastans* Dist., has been invariably mentioned as the pest of the cotton plant in the Punjab. We, however, found that amongst the samples collected from the cotton fields at Lyallpur, a few specimens of other species, not hitherto described, were almost always present. A collection of these specimens was sent to the Imperial Entomologist, Imperial Agricultural Research Institute, New Delhi, for identification and as a result of this, the following three new species have been brought to light:

(1) E. minor Pruthi,

(2) E. kerri var. motti Pruthi, and

(3) E. punjabensis Pruthi [ Pruthi, 1940 ].

In order to find out the extent to which the new species were present on cotton in different parts of the province, a survey was carried out during the last three years, the results of which are presented in this paper,

# SURVEY OF THE SPECIES

For the purpose of this survey, fortnightly collections of jassids were obtained from cotton fields from a large number of places. The help of the staff of the Punjab Department of Agriculture was sought for this work and the usual method of the hand-net and the killing bottle was employed. An effort was made to collect about 300 insects each time; but the actual number varied with the extent of incidence of the pest and the place of collection. At the time of the examination, the external morphological characters described by Pruthi [1940] did not enable us to identify the various species except E. devastans and, therefore, we had to resort to the only alternative of examining the male genetalia under the microscope. The collection was examined in two stages, firstly the separation of E. devastans Dist. from the rest of species and secondly the identification of the various other species.

PERCENTAGE OF E. DEVASTANS Dist. IN THE DIFFERENT PARTS OF THE PROVINCE

The percentage of E. devastans in the fortnightly collections made from different important cotton growing tracts of the province is given in Table I.

TABLE I

Percentage of Empoasca devastans Dist. in the jassid collection made from cotton during 1940-42

				JOEX	, k					AUGUST	ST					SEPTEMBER	MBER					OCTOBER	BER		
Localities surveyed				Fortnight	ight					Fortnight	.jpt					Fortnight	ght .					Fortnight	iight		
•	1	-				Ħ			1			п			н			П	ŀ		I			=	
	# <u></u>	1940 1941		1942 19	1940 18	1941 19	1942 18	1940 1	1941	1942	1940	1941	1942	1940	1941	1942	1940	1941	1942	1940	1941	1942	1940	1941	1949
Lyallpur .	<u>.</u>	100.0	· 9		.:	100.0 10	100.0	<del>-</del>	100.001	100.0		81.8	100.0		100.0	100.0		98.4	:	:	94.8	]:		0.66	<del></del>
Khanewal	•	·	· 		6-0	85 85		0.0	1:0	6.0	5.0		20-7	6-3	0:0	0.0	17.7	0.0	9.06	0.86	33.3	92.3	8.46	30-0	
Multan	•	76	. 6.92		un .	20-0	• • • • • • • • • • • • • • • • • • •	22.7	27.3	7.86 7.86	70.5	20.0	97.4	68-1	73.5	98.5	83.3	7.1.7	94-1	89.0	9.06	99.5	84.0		
Montgomery	• 1	94	94.8	:	•	82.1	14.8	<u> </u>	•	20.0	13.0	100.0	97.6	76-4	92.1	99-1	6.76	•	98.6	82.1		88.3	:	:	98.4
Bucheki .									:		•	•			100.0	100.0	:	100.0	100.0		100.0	100.0		100-0	
Shahpur		100.0			9 :	100.0 10	0.001	:	100.00	100-0		100.0	8.98		94.4	100.0	:	:		:	100.0	0.66	•		98.0
Sargodha .		96	96-3		1.6	50.8	96-3 6	0.89	98.7		27.8	93.3	94-1	6.86	100.0	92.4	92.0	98.4	6.06	87.0	98.3	:	:	87.3	
Bhalwal .					•	6-06	92.3 4	41.9	94.1	9.26	37.7	100.0	1.96	91.0	94.1	95.1	87.2	95.0	98.5	:	0.06	:	92.7	97.8	0.66
Chimiot .	•		9.78		<u>2</u> :	100.0	97-9		95-6	96-3	:	100.0	94.5		95.7	100.0	:	6.06	92.9	:	100.0	2-26	:	100.0	
Hansi .	•	<b>₹</b> ·			<u> </u>	100.0	:	80.1	100.0	93.0	2.76	94.7	8-66	8.26	100.0	9-86	6.96	8.86	98.6	92.7	92.3	9.66	:	7.9.7	
Јвапу .						33-3	69-2	25-0	0.00	0.02	£.63	2.29	0-06	98.5	91.7	6-56		8.96	0.00	74.0	91.5	95-5	:	9-88	
Chichawatni	-	100-0	<u>.</u>		<u></u>	55.8	65.0		÷.	\$-7.5°	:	711-4	84.1		6.06	91.7		8.76	27.27	:	95-4	73-3	:	83.58 63.58	
Hafizabad	•	: 	93.8		<u>-</u>	95.5	91.8	<del>-</del>	100.0	94.2	:	89.5	94.3		52.9	93.4	:	9.06	396-2	:	:	95.1	:	95.1	94.5
Arifwala .		3 <del>4</del> :	42.9				33.3	•	0.0+	<del></del>	:	87.5	12.5		55.6	80.0	•	8-22	2.29	:		85.7	:	33.3	
Nankana Sahib		100.0		•	<u> </u>	100.0	100-0		90.8	100-0		95.6	100-0		6.88	6.76	:	85.7	100.0	:		100.0		:	•
Vilhari .						0.55	•		1.8			57.1	:		83-3	•	:	75.0	:	:	50.0	:	:	88.89	
D. G. Khan	•				= :	100.0						0.0	:		030	:	:	2.99		•	:	:	:	:	
Sangala Hill				9	69.5			1:1:		,	96.9			0.80			9								

It will be seen from this table that the percentage of *E. devastans* was low to start with at most of the places and went on steadily increasing with the advance of the season, until towards the end it became the most predominating species. Another point of interest is that species other than *E. devastans* were much more in evidence in South West Punjab (Arifwala, Khanewal, Vihari and Multan) than in the rest of the province and it was a matter of great interest to find out the cause of this. This point will, however, be dealt with later.

# IDENTIFICATION OF OTHER SPECIES

The technique adopted for the indentification of species other than devastans was the same as described by Pruthi [1925]. It is evident that the identification of the species by this method is a slow and laborious process and it was, therefore, not possible for us to examine each individual in our collection. In order, however, to get a fairly accurate idea of the proportionate number of the various species, we selected at random 10 or 12 males of these species from each sample during 1940 and 1941 and subjected these to the microscopic examination. The samples of 1942 have not been examined, but there is no reason to believe that these would be different from the samples of the previous two years. The proportion of the various species is given in Table II.

It will be seen from these data that E. minor and E. kerri var. motti were the two most predominant species, the latter being more so than the former. It would also be seen that E. minor was found in greater abundance in Chiniot, Sargodha and Bhalwal, while E. kerri predominated in the rest of the province. It is unfortunate that with the present state of our knowledge of the alternative host plants of E. minor, we cannot assign any reason for the relative preponderance of this species in these three places. It must, however, be noted that these places lie adjacent to one another and have similar soil and climate and are mostly inhabited by the Jungle\* Tribe, who have very rigid social customs and have the habit of growing large areas under fodders [ Khan, 1938 ]. It is probable that E. minor is a pest of some fodder crop, and comes on to cotton in the absence

of its favourite host. This point, however, needs verification by careful observation.

The causes which led to the comparative abundance of *E. kerri* var. motti on cotton in early part of the season in certain localities and its virtual absence at the end of the season was the next point needing investigation. For this purpose a survey was made of the standing crops where cotton was growing and it was found that guard (Cyamopsis psoralioides) harboured a large number of jassids. The attack on this crop is really very heavy every year and *E. kerri* var. motti is found on it practically to the exclusion of every other species. It was, therefore, argued that cotton was only an alternative host plant of this insect, which preferred guara to cotton.

As is well known, guara is grown both for fodder and as a green manure crop. In the later case, the crop is allowed to grow rather luxuriantly and is buried in the soil any time before 15 September. When the crop is buried, the adult jassids then present on it migrate to other food plants. Thus in fields of cotton situated near guara, there is always a possibility of a larger number of these species being present than if no guara was grown near about. Also when guara is buried all the adult population will transfer itself on to cotton. The figures given in Table III are interesting in this connection.

Guara was buried during the interval between the first and second date of sampling during August and the drop in the percentage of E. devastans during this period can be explained by the invasion of E. kerri from guara to cotton. This drop was much more marked in the field situated near the guara crop than in the one away from it.

It is well known that guara as a green manure crop is grown in much larger proportions in the districts of Montgomery and Multan than in Lyallpur. At the British Cotton Growing Association Farm, Khanewal, from where the samples noted in Table I were taken, guara is grown on at least 2.5 acres per square (25 acres) every year, and this may explain the comparative abundance of E. kerri on cotton in the early part of the season at Khanewal.

It will also be clear from Table III that the comparative abundance of *E. kerri* var. *motti* did not continue for more than a fortnight after the removal of *guara*. In order to elucidate this point, experiments on oviposition and the development of

<sup>\*</sup>The original inhabitants of the Canal Colonies of the Punjab. They are tall, well-built and always maintain a big herd of milch cattle.

E.P. = Empowsca punjabensis

Proportion of different species of jassids collected from cotton during 1940 and 1941

Table   Fortsmeth   Fortsmet	SEPTEMBER   Sept	B.P. B.M. B.K. B.P. E.M. B.K. B.P. B.M. B.M. B.M. B.M. B.M. B.M. B.M
OCTOBEA  III  IX. E.P. E.M. E.K. E.P. E.M.  O	CCTOBER   CCTO	Totaleta   Totaleta
Portuight    1   3   0   0	Fortuight   Fortuight   Fortuight   Fortuight   Fortuight   F.K. E.P. E.M. E.K. E.P.   F.M. E.K. E.P. E.M. E.K. E.P.   F.M. E.K. E.P. E.P. E.M. E.K. E.P. E.M. E.M. E.M. E.M. E.M. E.M. E.M	A TOCTOBER.  Fortnight  La.M. B.K. B.P. E.M. E.R. B.P. E.M. B.C.  1 3 0 0 2 0  0 2 0  1 1 3 0 0 0 2 0  4 17 0 3 18 0 1   1 10 0 0 1 0  1 11 0 1 6 0  1 11 0 1 6 0  1 11 0 1 6 0  1 11 0 1 6 0  1 11 0 2 0  1 11 0 2 0  1 11 0 2 0  1 11 0 2 0  1 11 0 2 0  1 11 0 2 0  1 11 0 2 0  1 11 0 2 0  1 11 0 2 0  1 11 0 2 0  1 11 0 2 0  1 11 0 2 0  1 11 0 2 0  1 11 0 2 0  1 11 0 2 0  1 11 0 2 0  1 11 0 2 0  1 11 0 2 0  1 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
	H H H H H H H H H H H H H H H H H H H	H

N.B.-E.M.=Empoasca minor

E.K. = Empoasca kerri var. motti

TABLE III

Percentage of E. devastans Dist. in fortnightly collections from fields of cotton near and away from guara in 1941 at Lyallpur

	JULY			AUGUST			SEPTEMBER			OCTOBER						
	forti	[ night	I fortn	I light	I fortn	ight	I fortn	I ight	fort	I night	I forti	I night	J fortn	l night	fort	II night
	Total	%E.D.	Total	%E.D.	Total	% <b>E.</b> D.	Total	%E.D.	Total	%E.D.	Total	%E.D.	Total	%E,D.	Total	%E.D.
1. Field of cotton contiguous to guara field	77	100.0	165	100.0	319	97.8	306	18.0	256	99-2					••	
2. Field of cotton 1½ miles away from guara field	117	100.0	145	100.0	180	100.0	292	31.8	295	100•0	307	98-4	306	94.8	304	99-0

the nymphs of the two species, *E. devastans* and *E. kerri* var. *motti* on *guara* and cotton were carried out during 1942. The results of this investigation are summarized below:

Seventy-two adults, both males and females, of *E. devastans* and *E. kerri* var. *motti* were collected and liberated on young leaves of *guara* and cotton and the number of nymphs hatched out were later on counted. The necessary precautions about the absence of oviposition on the particular leaves previous to the liberation of the adults were carefully observed. The figures are given in Table IV.

Table IV

Oviposition of E. devastans and E. kerri var. motti
on cotton and guara

Species of jassids liberated	Plant on which liberated	No. of adults liberated	No. of nymphs hatched
E. devastans	Guara	72	Nil
E. devastans	Cotton	72	46
E. kerri	Cotton	72	3
E. kerri	Guara	72	269

It is seen from Table IV that *E. kerri* var. motti laid very few eggs on cotton while *E. devastans* was not at all able to lay eggs on guara. *E. kerri* was able to breed very freely on guara and this may be the reason of very large population of this pest found on guara every year.

Another experiment in which the development of the nymphs of these two species on cotton and guara was studied was performed during 1942. A certain number of first instar nymphs were liberated on young leaves and were carefully watched. The number of adults that finally emerged was also noted. In this case again, only these young leaves which had been previously

sleeved to prevent eggs being laid on them were used. The figures are given in Table V.

Table V

Development of the nymphs of E. devastans and E. kerri var. motti.

Species of jassid liberated	Plant on which liberated	No. of nymphs liberated	No. of adults which emerged
E. kerri var. motti .	Guara	165	81
Ditto	Cotton	150	71
E. devastans	Cotton	150	80
Ditto	Guara	150	15

From Tables IV and V it will be seen that E. kerri var. motti lays very few eggs on cotton (and possibly the percentage of hatching is also very low), but the young nymphs have no difficulty in feeding on cotton and reaching maturity.

Here then, we have an explanation of the phenomenon of the rise in the number of *E. kerri* var. *motti* on cotton after the removal of *guara*. The adults of this species invade the cotton fields near *guara*, but the adults all die out soon after. As very few eggs are laid the species practically disappears from the cotton crop within a fortnight.

Small scale cage experiments, in which *E. kerri* var. motti was liberated on cotton plants in very large numbers to keep up a high degree of infestation, were performed during 1941 and 1942. It was invariably noticed that the death rate of the insect in the cages was very high and, therefore, daily liberation of a large number of fresh adults was necessary to maintain a high degree of infestation. It was, however, noticed that inspite of the high degree of infestation maintained artificially inside the cage, the characteristic symptoms of the attack of *E. devastans*—crinkling and reddening of leaves—did not develop on the plants.

Although no data on the loss in yield or deterioration in quality of fibre are available, it is considered that such losses cannot be high and, therefore, E. kerri var. motti should not be considered as a serious pest of the cotton plant in the Punjab.

It will also be seen from Table II that very few specimens of E. punjabensis were collected from cotton. It is suggested that this species is not as pest of cotton at all and the stray specimens recorded were only casual visitors.

### SUMMARY

Amongst the new species of jassids recently discovered it was found that E. kerri var. motti and E. minor were most commonly found on cotton plants in early part of the season and E. punjabensis was perhaps only a casual visitor to the cotton

To the list of different species of jassids attacking cotton compiled by Afzal Hussain and Lal [1940] the name of E. minor may now be

So far as our present knowledge goes, both the new species mentioned above are not injurious to the cotton plant which is only their alternative host. The most favoured host plant of E. kerri is guara, and that for E. minor yet awaits determination.

#### ACKNOWLEDGEMENT

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# ON THE BIOLOGY OF THE VEGETABLE MITE (TETRANYCHUS CUCURBI-TAE RAHMAN AND SAPRA: FAM. TETRANYCHIDAE)

By Khan A. Rahman and Amar Nath Sapra, Entomological Laboratory, Punjab Agricultural College and Research Institute, Lyallpur

(Received for publication on 23 November 1944)

(With Plates I & II and ten text-figures)

The vegetable mite was first collected in November 1934 from desi sem (Canavalia ensiformis) at Lyallpur and was identified as a new species of the Genus Tetranychus by Mr R. J. Whitick of the British Museum and was described by Rahman and Sapra [1940,1] as Tetranychus cucurbitae.

T. cucurbitae is a very serious pest of vegetables and other crops in the Punjab and in view of its importance, a detailed study of its life history and habits was made and the results are presented in this paper.

# TECHNIQUE

For a study of the biology of this small-sized Arthropod, special technique had to be adopted. Therefore, the methods employed by McGregor and McDonough [1917] for a study

of mites and Floyd-Smith [1931] for confining thrips on the leaves of the host plants, were tried, but none of these methods proved successful. On the other hand, the apparatus evolved by Storey [1928] for a study of the insect-vectors of plant viruses was, after some modification, found to be useful for studying the biology of this mite. This is briefly described below.

The apparatus (Fig. 1) consists essentially of four parts: two short tubes (a) and (b), a semicircular steel wire ring (c) which serves as a spring and glass tube (d) for confining mites. The two tubes (a, b) are formed at either extremity of a steel bar (e). A bamboo rod is passed through (a) and is fixed to the ground and the height at which the apparatus is to be adjusted is regulated with the screw(s).

One end of the ring (c) is soldered along the length of the tube (b), while its other end bears a cork which is provided with a padding of cotton-wool tied to it with muslin and this serves as a buffer for the leaf of the host plant. Through (b) passes the glass tube (d) and its lower end rests on the leaf over the padding. The edge of (d) which touches the leaf surface is thoroughly ground to make it smooth. The height of the tube is adjusted with the screw (ss) to secure correct pressure between the edge of the tube (d) and the leaf, between which is interposed a rubber ring to avoid injury to the latter by the former and to stop the escape of mite through loose contact of the two.

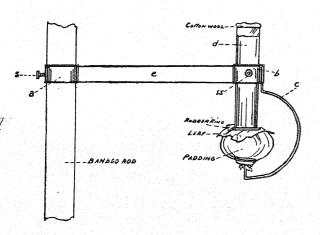


Fig. 1. Microcage

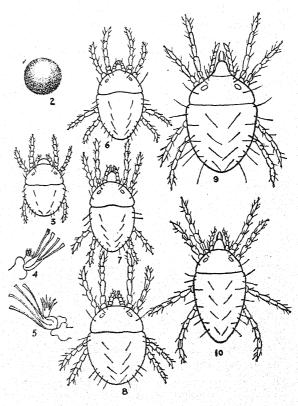
The tubes (a) and (b) are 1.8 cm. in length and 2.5 and 2.0 cm. in diameter respectively, the bar between them being 12.5 cm. long. The glass tube (d) is of 7.0 cm. length, 1.1 cm. internal diameter and 0.1 cm. thick walls.

Desi sem (Canavalia ensiformis) was used as host plant and it was grown under cover of thatching. The crop is in the field from April to December. The cover was essential as the mites could not stand direct sun, which made them restless and were generally lost in the cotton-wool plug on the upper end of the tube. Moreover, the afternoon temperature inside the glass tubes in the open, always, proved fatal for the mites after the middle of April.

### DESCRIPTION OF VARIOUS STAGES

Egg (Fig. 2). Spherical, 0.112 mm. in diameter, transluscent when freshly laid and appears

like a drop of honey in the web. Colour gradually changes to brown assuming a deeper tinge before hatching, when the area along the periphery becomes transparent and red eye-spots become visible.



Figs. 2-10

The vegetable mite (Tetranychus cucurbitae R. & S.)

Fig. 2. Egg ×91; Fig. 3. Larva ×91; Fig. 4. Tarsal claw (male) larva; Fig. 5. Tarsal claw (female) larva; Fig. 6. Male protonymph ×91; Fig. 7. Female protonymph ×91; Fig. 8. Deutonymph ×91; Fig. 9. Adult female×91; and Fig. 10. Adult male ×91.

Larva (Fig. 3). Length 0.140-0.196 mm., width 0.126-0.154 mm. Almost spherical in outline and light amber in colour at hatching, becoming elongated later on and developing a greenish tinge and dark specks dorsolaterally with feeding. It has three pairs of legs.

Male and female larvæ distinguishable at

Male and female larvæ distinguishable at birth, the tarsal claws of the first pair of legs, as in adults, ending in a whorl of teeth in case of male larvæ (Fig. 4) and in six hair like processes in female larvæ (Fig. 5). These characters persist in the later stages as well.

Protonymph (Figs. 6 & 7). Different from larva in being slightly bigger and with longer bristles on the dorsum and with four pairs of legs. The colour changes to deep green and dark specks on dorsum increase in size. Towards the end of this stage, the male and female protonymphs distinguishable apparently: male ones (Fig. 6) 0.210-0.290 mm. long and 0.161-0.175 mm. broad and more or less elongated while the female ones (Fig. 7) 0.210-0.280 mm. long and 0.162-0.182 mm. broad and more or less ovate.

Deutonymph (Fig. 8). Length 0.276-0.341 mm., breadth 0.198-0.240 mm. Only females pass through this stage, different from the previous stage in being bigger and genetalia being visible.

Adults. (Figs. 9 and 10). Have been described in detail elsewhere [Rahman and Sapra,

1940,1].

#### DISTRIBUTION AND FOOD PLANTS

Tetranychus cucurbitae has, so far, been recorded from Lyallpur, Gujranwala, Montgomery, Lahore and Sargodha districts of the Punjab, but because of the wide range of its host plants, it is most probably very widely distributed in the province. It has been collected from the following plants of which the first 13 suffer the most from its ravages: tinda (Citrullus vulgaris var. fistulosus), water melon, melon, different varieties of gourd, ghia tori (Luffa aegyptiaca), tomato, desi sem (Canavalia ensiformis), hollyhock, brinjal, cabbage, mash, mong, moth (Phaseolus spp.), peas, knoll-khol, cauliflower, arhar (Cajanus indicus), potato, onion, arum, poppy, swedes, turnips, brussel sprout, radish, cotton, sunkukra (Hibiscus cannabinus), lady's finger, different varieties of citrus, darek (Melia azedarach), alyer (Dodonaea viscosa), jantar (Sesbania sp.), sheesham (Dalbergia sissoo), berseem, shaftal, lucerne, senji (Melilotus parviflora), maina (Medicago denticulata), maithey (Trigonella Foenum graceum), soybeans (Glycine hispida), cow peas, sunn-hemp, rose, crab-apple, karela (Momordica charantia), celery, dhodhak (Sonchus sp.), sunflower, chandni (Tabernaemontana coronaria), leli (Convolvulus arvensis), railway creeper (Ipomaea sp.), petunia, dodhak (Euphorbia sp.), mulberry, clarkia, and sweet potato.

#### LIFE HISTORY

The males emerge a little earlier than the females and wander about on the leaf in search of a female. On coming across a resting deutonymph the male places its anterior pairs of legs on it and waits for its emergence, there being more than one males resting by a deutonymph. Its sexual behaviour and mode of mating resembles that of *Paratetranychus indicus* Hirst [Rahman and Sapra, 1940,2].

During April-September, the males generally die within 24 hr. after mating, but a few of them may live up to 48 hr. The unmated ones, however, live for 5-8 days during April-September and 9-20 days during March and October-December; the females, on the other hand, live for 10-12 days and 14-25 days during the corres-

ponding periods respectively.

Pre-oviposition period. Since the females are fertilized as they emerge, the pre-copulation period is negligible. The pre-oviposition period, however, varies with the temperature from less than 24 hr. (May-September) to 96 hr. (February-April and October-mid.-December), while the females may not oviposit even for a fortnight during the period from middle of December to end of January.

Oviposition. The eggs are laid at random in the web generally on the lower surface of the leaves from the beginning of March to the middle of December, the largest number being laid during April-October. The oviposition period lasts for 8-10 days during April-September and 12-20 days during March and October-December. During January-February eggs are laid very rarely, when there are long spells of bright sunny weather. The number of eggs laid is different for mated and unmated females. So the rate of oviposition varies with a temperature and the type of the female. The mated females laid a maximum of 13 eggs daily, the total number of eggs laid by them in their life time being 61-93 with a maximum during May-June and August-September, while the unmated ones laid a maximum of 9 and total of 33-59, the maximum being during April-June and August-September (Table I).

Post-oviposition period. The females, generally, die immediately after laying the last egg, this period extending to a maximum of 24 hr. during April-September, and 2-5 days during

March and October-December.

Table I

Oviposition records of T. cucurbitae R. & S.

			Nu	mber of eggs	laid by			
		Mate	ed females		Unmated females			
Month		Total			Total			
	Max.	Min.	Average	Per day	Max.	Min.	Average	Per day
March April May June July August September October November December	80 86 93 90 84 90 89 79 80 81	66 65 61 78 71 75 72 66 61 63	75·0 77·5 79·8 85·0 77·6 82·0 82·3 74·7 72·1 72·0	1—4 5—12 10—13 7—12 6—12 7—11 8—10 6—8 1—5 0—3	50 57 56 59 50 54 58 54 52 50	33 43 42 45 45 47 47 44 37	44·0 52·0 50·8 52·3 47·6 51·3 51·8 49·3 46·0 45·2	1—3 5—8 5—9 4—9 5—8 4—9 3—6 2—4 1—3 0—2

Hatching. The chorion splits on one side along the circumference in a vertical plane. The larva widens this opening by pushing the two sides apart with its legs and comes out, leaving the egg-shell intact on the leaf surface.

The egg hatching is almost cent percent excepting from end of December to middle of February, when a majority of them fail to hatch.

The duration of the egg-stage varies with season: eggs laid from middle of April to middle of September hatch in  $2\frac{1}{2}$ -3 days, those laid in March to middle of April and middle of September to end of November, hatch in  $3\frac{1}{2}$ -12 days, while those laid in January-February hatch in 30 days (Table II).

TABLE II Egg stage of T. cucurbitae R. & S.

Egg laid on	Egg hatched on	Duration (in days)
10-I (M)	9-II (M) .	30
12-ΠΙ (M) .	20-III (M) .	8
20-IV (E)	23-IV (E)	3
21-V (È)	24-V (È)	3
25-VI (É)	28-VI (É) .	3
28-VII (É) .	31-VII (M) .	$2\frac{1}{2}$
30-VIII (É) .	2-IX (E)	3
29-IX (E) .	3-X (M)	$3\frac{1}{7}$
8-X (È)	13-X (M)	$4\bar{3}$
21-X (M)	27-X (M)	$ ilde{6}$
7-XI (M) .	14-XI (É)	71
30-XI (M) .	12-XII (M) .	12

(M) = Morning and (E) = Evening

The female mite passes through the Instars. following instars-egg: active larva-quiescent larva: protonymph-quiescent protonymph: deutonymph-quiescent deutonymph, the duetonymph instars being absent in case of male. When the larva or the nymph attains its growth, it fixes itself by thrusting its stylets into plant tissue and enters resting (quiescent) stage. During this period of rest, it neither feeds nor moves and its anterior pairs of legs lie parallel to each other and the rostrum. This stage is a sort of preparatory phase to the next stage. Its duration in each of the instars lasts for a few hours during March-November and for 1-3 days during December and 2-3 days during January-February.

The duration of the immature stages varies with the season: they separately occupy  $1\frac{1}{2}$ -2, 3-6,  $7\frac{1}{2}$ -9 and 20 days in males and  $2\frac{1}{2}$ -3, 4-9,  $10\frac{1}{2}$ -13 and 27 days in case of females during May-September, March-April and October, November-December and January-February, respectively.

Almost all the larvae hatching out from the eggs mature into adults, except about 10 per cent of them fail to moult successfully during May-June, especially in the first instar.

Life cycle. The generations overlap in nature and all stages of the mite are available during March-December. Under controlled conditions, as many as 32 generations were counted in course of a year.

TABLE III

Duration of immature stages of T. cucurbitae

Egg	Adults en	Duration (in days)		
hatched on	hatched on Male Female		Male	Female
9-II (M)	1-III (M)	S-III (M)	20	27
20-III (M) 6-IV (M)	26-III (M) 9-IV (M)	29-III (M) 11-IV (M)	6	9 5
23-IV (E) 24-V (E)	26-IV (E) 26-V (M)	27-IV (E) 27-V (M)	$1\frac{3}{2}$	$2\frac{1}{2}$
28-VI (E) 11-VII (E)	30-VI (E) 13-VII (M)	1-VII (E) 14-VII (M)	$1\frac{2}{2}$	$2\frac{1}{2}$
19-VIII (E) 17-IX (M)	21-VIII (E) 19-IX (M)	22-VIII (E) 20-IX (M)	2	3
2-X (M) 27-X (M)	6-X (E) 2-XI (M)	7-X (E) 4-XI (M)	$\frac{3\frac{1}{2}}{6}$	41 8
14-XI (E) 12-XI (M)	22-XI (M) 21-XI (M)	25-XI (M) 25-XI (M)	$\frac{7\frac{1}{2}}{9}$	$\frac{10\frac{1}{2}}{13}$

(M) = Morning and (E) = Evening

The duration of the life-cycle depends upon the temperature. It occupied 4-7, 8½-12, 15-21 and 50 days in males and 5-9, 10-17, 18-25 and 57 days in females when the temperature °F. varied between 60·2-107·0, 52·2-88·6, 40·2-77·6 and 39·0-71·3, respectively (Table IV).

Table IV Duration of life-cycle of T. cucurbitae R. & S.

Eggs	Adults en	nerged on		ration days)	Temperature °F.	
laid on	Male	Female	Male	Female	Min.	Max.
10-I (M)	1-III (M)	8-III (M)	50	57	39.0	71.3
12-III (M)	26-III (M)	29-III (M)	14	17	52.2	83-6
2-IV (M)	9-IV (M)	11-IV (M)	7	9	60.2	91-7
20-IV (E)	26-IV (E)	27-IV (E)	6	7	70.2	105-5
7-V (E)	12-V (E)	13-V (M)	5	5 <u>1</u>	81.0	106-5
21-V (E)	26-V (E)	27-V (M)	41	5 <u>1</u>	80.2	107-0
10-VI (E)	16-VI (M)	17-VI (M)	5분	61/2	76.1	93.1
25-VI (E)	30-VI (E)	1-VII (E)	5	6	78.0	96.2
9-VII (M)	13-VII (M)	14-VII (M)	4	5	83-3	104.3
28 <b>-VII (E</b> )	2-VIII(M)	3-VIII(M)	41/2	51	83.7	98-0
16-VIII(E)	21-VIII(E)	22-VIII(E)	5	- 6	77.5	96-0
30-VIII(E)	4-IX (E)	5-IX (E)	5	6	78-8	95.5
14-IX (M)	19-IX (M)	20-IX (M)	5	6	71.8	98-5
29-IX (E)	6-X (E)	7-X (E)	7	8	68-6	91.7
8-X (E)	17-X (M)	18-X (E)	81	- 10	59.7	88-6
21-X (M)	2-XI (M)	14-XI (M)	12	14	53-9	82.5
7-XI (M)	22-XI (M)	25-XI (M)	15	18	45.8	77:6
30-XI (M)	21-XII (M)	25-XII (M)	21	25	40.2	69-6

(M) = Morning and (E) = Evening

#### WEB FORMATION

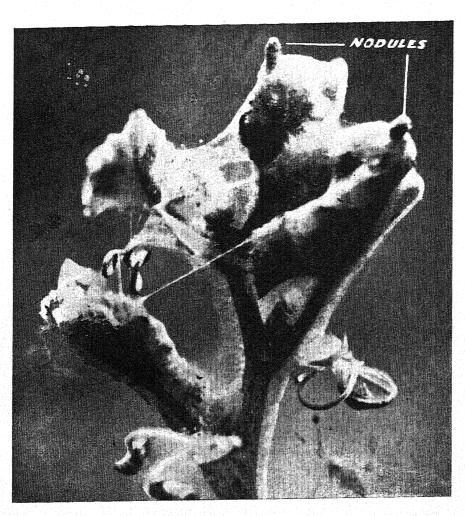
The females, when on fresh leaves, select a concave area generally between two veins, where they spin web and there may be large number of small patches of the web spun by different females. With the increased activity, these patches extend and coalesce with each other with the result that there is ultimately a single sheet of web covering the entire surface. The web formation is at first confined to surface initially infested, but is latter carried on indefinitely and on all sides and the web assumes the shape of a thick sheath covering the entire leaf (Plate II).

### DISPERSAL

The females are responsible for fresh infestations. Before settling down on a leaf for feeding, spinning and ovipositing, they move about on and along the veins or edges of the leaves, from where they are easily dislodged by gusts of wind and carried to fresh plants. These mites have the interesting habit of collecting in nodules (Plate I), each of which may harbour as many as 1198 freshly emerged females, at the peak of the plant and thus expose themselves to the action of wind for dispersal. Whenever a blade of grass touches the infested plant, the mites collect at its apex. In the same field, the mites migrate from one leaf to the other through the contact of the two.

#### SEASONAL HISTORY

In the beginning of March, when the season warms up, the overwintered females become active and begin to lay eggs, the first generation being completed in about three weeks. Later generations follow in quick succession. Some individuals get carried to healthy plants through contact or by wind and they start fresh infestation. By end of April the pest is available on majority of the food plants mentioned above. This is followed by a period of maximum activity up to July when the monsoon rains kill all stages except the eggs, which produce future progeny. After cessation of rains the mites multiply rapidly and again become a pest in September-October. With the fall in temperature after October the population gradually declines, till by the middle of December mostly gravid females remain,



Mode of dispersal

Web intact

Web removed

which overwinter. These females feed and do sometimes lay eggs whenever the season becomes warmer. In all 32 generations are gone through during the course of year and those during March-December overlap.

The annual calendar of the activities of this pest is as under:

- 1. March-April—Quick multiplication on weeds, cabbage, knoll-khol and holly-hock; migration to other plants.
- 2 May-mid. July—Activity at its zenith on large variety of plants especially
- cucurbits and other vegetables.
  3. Mid. July-August—Mites rare except the eggs, available on cucurbits and sweet potato.
- 4. September-October—Again active especially on *linda*, desi sem and pulses.
- 5. November-mid. December—Population on decline, available on cauliflower, cabbage, knoll-khol and desi sem.
- 6. Mid. December-February—Mostly gravid females on cabbage, knoll-khol, holly-hock and weeds.

### PARTHENOGENESIS

Parthenogenesis is common in this species also, the parthenogenetically developed progeny consisting of males only. The fertilized females, however, give rise to a progeny, which has about 80 per cent females.

# SEX RATIO

The sex ratio is 50: 50 from May to September. In October the number of males goes up, but gradually declines during the subsequent months, so much so that the males are practically absent during January. Due to the paucity of males during December-January the majority of females remain unmated and reproduce parthenogenetically during March-April and thus the male population is higher during these months.

Table V gives the sex ratio in different months. The counts were made on ten leaves of holly-hock, tinda, ghia-tori, or lady's finger in the later half of every month.

TABLE V
Sex ratio in T. cucurbitae R. & S.

Month	No. of males	No. of females	M : F
January February March April May June July August September October November December	1 27 243 481 306 338 63 85 69 123 25	209 428 130 285 323 370 81 90 84 90 111 166	0.5 : 99.5 6 : 94 65 : 35 61 : 39 49 : 51 48 : 52 44 : 56 49 : 51 46 : 54 58 : 42 19 : 81
		100	2:98

# NATURE AND EXTENT OF DAMAGE

The mites suck plant sap with their stylets and their places of feeding appear as white spots on the leaf surface. With the increase in the intensity of attack the white spots increase in number and gradually coalesce with each other finally producing large patches. The leaves loose their green healthy colour (Plate II), gradually wilt, dry and fall off. This decreased vitality and leaf drop effect growth, flowering and fruiting and a poor or no crop results. In some of the host plants, especially desi sem (Canavalia ensiformis), the damaged portions of the leaves become rust-red in colour.

In severe cases of infestation, the web spun by these mites is profuse and very thick (Plate II) and covers the foliage on all sides. The dust particles blown by wind during May-June, when mite attack is at its worst, are deposited in the web. The web together with the soil particles smothers the leaves and this was tested experimentally: leaves of tinda (Citrullus vulgaris var. fistulosus) were infested with 25 gravid females in the beginning of June, in some cases the web spun by the mites was broken daily, while in others it was left to develop undamaged, and it was seen that the leaves dried in 19 days where the web was not broken and 28 days where the web was broken. In most of the cases the attacked plants do not bear any rruit.

# SUMMARY

Tetranychus cucurbitae is the most serious and commonest of the recorded plant mites. It has been collected from about 55 plants and because of the wide range of its hosts, it is probably ex-

tensively distributed in the province.

The technique for confining mites individually on the leaves is described and description of the immature stage is given. It is shown that the duration of oviposition, pre-oviposition and postoviposition periods as well as that of egg and larval stages depends upon the season. During the active period (April-mid. October) the life cycle is completed in 4-81 days in males and 5-10 days in females. The mated and unmated females laid 61-93 and 33-59 eggs respectively and progeny from unfertilized eggs comprised of males only. The seasonal history calendar is given and it is shown that the damage by the pest is at its peak during May-mid. July

The pest has September-mid. October. and been observed to go through 32 overlapping generations during the course of a year and the females predominate except during March-April and October.

The pest is distributed through freshly emerged females and mode of dispersal is described. The attacked leaves loose green colour, dry and fall off and the attacked plants bear no or poor fruit.

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#### DIFFERENT NATURAL AND ARTIFICIAL RELATIVE AVAILABILITY OF PHOSPHATES IN CALCAREOUS SOILS

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(With Plate III)

PHOSPHORUS is one of the most important of the principal plant-food materials in which Indian soils are usually deficient. Taking India as a whole there are sufficient indigenous supplies of phosphatic manures from all sources consisting of raw mineral phosphates, such as Trichinopoly nodules and apatite, bones, and fish guanos to meet the present demands, although there are isolated localities where the question of the supply of superphosphate appears to be important. The superior advantage of compound fertilizers like ammophos, diammonphos, leunaphos, niciphos, etc., recently appearing in the market under various trade names, is to be ascribed to their additional constituent of nitrogen besides phosphorus.

Calcareous soils in the Gangetic alluvium around Pusa in Bihar contain from 30 to 40 per cent of They are particularly carbonate. deficient in available phosphates for most of the common agricultural crops and respond well to phosphatic fertilizers under suitable cultural conditions. Therefore, in order to determine the relative availability of different natural and artificial phosphates, a series of pot experiments was carried out in the winter of 1933 with Pusa calcareous soil containing 33 per cent of calcium carbonate.

Four pots of similar dimensions, 9 in. in diameter and 12 in. high, formed a group and received similar treatment. Each pot contained 15 kilos of air-dry soil and 16 per cent of moisture was maintained in the soil throughout the experiment. A basal dressing of potash and nitrogen was given to all the pots at the rate of 80 and 100 lb. per acre as sulphates of potassium and ammonium respectively. Allowance was made for either nitrogen or potash contained in bonemeal, ammonium or potassium phosphate. Different phosphates were applied to different groups of pots at the rate of 50 mg. of P2O5 per kilo of soil or 100 lb. of P2O5 per acre. There was a group of control pots for comparison, where no phosphate was added. Bonemeal used was obtained by treating raw bones with a solution containing 1.5 per cent caustic soda and 1 per cent common salt for 70 days and then crushing them fine after washing with water and drying. Apatite and Trichi-nodules were finely pulverized.

Mustard was sown on 11 November 1933 and the crop harvested on 6 March 1934. The mean yields of grain are given in Table I along with their statistical examination by Fisher's [1932] analysis of variance.

TABLE I

The relative availability of different phosphates as reflected in the yield of mustard in a calcareous Pusa soil and their classification on the basis of their yields

Group	Phosphatic fertilizers	Mean yield in gm.	Per cent increase over control
I	Control	6.05	
II	Aluminium phosphate, AlPO <sub>4</sub> , 3H <sub>2</sub> O	7.18	19.0
	Apatite	7.40	22.3
	Trichi-nodules	8.00	32.2
	Bonemeal	8.28	36.8
	Potassium phosphate, KH <sub>2</sub> PO <sub>4</sub>	8.40	38.8
	Sodium phosphate, Na <sub>2</sub> HPO <sub>4</sub> , 12H <sub>2</sub> O	8.50	40.5
III	Magnesium ammonium phos- phate, Mg NH <sub>4</sub> PO <sub>4</sub> , 6H <sub>2</sub> O	9.45	56.2
	Iron perphosphate, FePO <sub>4</sub> , 2H,O	9.50	57.0
	Dicalcium phosphate, CaHPO <sub>4</sub> , 2H <sub>2</sub> O	9.78	61.6
	Tricalcium phosphate,	9.85	62.8
IV	Ammonium phosphate, $(NH_4)_2HPO_4$	12.40	105.0
	Superphosphate	13.75	127.3

From Table I it is evident that all the treated pots have produced higher crop returns than the control according to the quality of the phosphatic fertilizer employed. The differences in mean yields of grain between the control and every other treatment are highly significant being greater than the critical value of difference except in the cases of apatite and aluminium phosphate. The higher crop yields with these two fertilizers were not significant. Trichi-nodules give significant results for 5 per cent, and the rest even for one per cent level of significance. Superphosphate ammonium phosphate give individually the most significant results over the rest, but between them there is no significance. Thus, except apatite and aluminium phosphate, all the other phosphatic fertilizers are made available in calcareous soils and can effectively supply the phosphatic nutrition of plants.

The fertilizers may be classified into four broad groups according to the magnitude of the yields obtained, as shown in the first column of Table I.

Eleusine coracana (vernacular ragi, marwa) was grown as the next kharif (summer) crop in 1934 in these very pots in order to study the residual effect of these fertilizers. Only nitrogen at the rate of 50 mg. per kilo of soil as ammonium sulphate was renewed in all the pots, but no potash or phosphates were added. Eight seeds of ragi were sown in each pot on 14 June 1934. A month after, the ragi seedlings were thinned to four in each pot. The rejected seedlings numbering 12 from each group of pots were weighed in order to find out the variation in growth under different phosphatic fertilizers. The results are set forth in Table II.

Table II

Fresh weight of ragi seedlings of about four weeks' growth under different phosphatic fertilizers

Original treatment	Fresh weight in gm.	Per cent increase or decrease over control
Control	5.10	
Bonemeal	9.60	+88.2
Apatite	6.40	+25.5
Trichi-nodules	3.60	-29.4
Magnesium ammonium phos- phate	<b>4·4</b> 0	-13.7
Tricalcium phosphate	10.40	+104.0
Dicalcium phosphate	7.15	+40.2
Superphosphate	9.50	+86.3
Ammonium phosphate	15.30	+200.0
Sodium phosphate	21.25	+316.7
Potassium phosphate	12.70	+150.0
Iron perphosphate	3.70	-27.5
Aluminium phosphate	7.15	+40.2

From Table II it is evident that except Trichinodules, iron and magnesium ammonium phosphates, all the other phosphatic fertilizers have produced better vegetative growth than the control. All the water-soluble fertilizers except superphosphate have given the best residual effect. Next comes in order the group representing super, bonemeal and tricalcium phosphate, and last of all are apatite, aluminium and dicalcium phosphates.

This grouping of the fertilizers for the residual effect does not, however, exactly fit into their classification as shown in Table I for the primary

effect except in the case of tricalcium and ammonium phosphates. Aluminium phosphate and apatite whose primary effect was not significant on the yield of mustard, have shown an appreciable residual effect. On the other hand, Trichinodules, iron and magnesium ammonium phosphates, whose primary effect was considerable, have not only failed to show any residual effect, but produced a lesser vegetative growth than even the control.

A photograph of ragi seedlings of about seven weeks' growth taken on 4 August 1934 from representative pots is given in Plate III, fig. 1. It indicates the variation in growth due to different phosphatic fertilizers from left to right in the order given in Table II.

This variation in vegetative growth was not, however, reflected in the mean yields of grain given in Table III, when the crop was harvested on 24 September 1934.

TABLE III

The residual effect of different phosphatic fertilizers as reflected in the yield of ragi grain in Pusa Calcareous soil

Original treatment	Mean yield in gm.	Percentage variation over - control
Control	12.80	
Bonemeal	10-13	20.9
Apatite	14.93	16.6
Frichi-nodules	12.95	1.3
Magnesium ammonium phos- phate	13.68	7.0
Pricalcium phosphate	21.68	69.4
Dicalcium phosphate	16.25	27.0
Superphosphate	19.35	51.2
Ammonium phosphate	17.48	36-6
Sodium phosphate	22.60	76.6
Potassium phosphate	20.80	62.5
Iron phosphate	20.30	58.6
Aluminium phosphate	22.53	76.0

Standard error, for comparison of mean yields . . . =1.095
Critical difference for 1 per cent =2.97
Critical difference for 5 per cent =2.22

From Table III it is evident that all the phosphates except bonemeal have produced a higher crop return than the control. The differences in mean yields of grain between the control and every other treatment are highly significant being greater than the critical value of difference

even for one per cent level of significance except in the case of bonemeal, apatite, Trichi-nodules, and magnesium ammonium phosphate. Among the latter, bonemeal gave altogether negative results when compared with the control and the rest slightly higher yields which were not significant. Aluminium phosphate whose primary effect on mustard was not significant, gave about the best residual effect showing that it was rendered available in the soil after some lapse of time. On the other hand, Trichi-nodules, bonemeal, and magnesium ammonium phosphate, whose primary effect was appreciable, showed a marked deterioration and produced a poor yield of ragi. Considerable residual effect of the rest of the fertilizers is, however, manifested in the higher crop yields than the control. Iron and tricalcium phosphates have uniformly maintained their good effect in both the crops. Sodium and potassium phosphates have better residual effect than the primary, but the reverse is the case with super and ammonium phosphate.

The solubility of phosphatic fertilizers in a 2 per cent solution of citric acid is considered to be a measure of their availability in a soil. It was determined in order to find out its relationship with crop yields, if any. The results are arranged in Table IV according to the increasing order of solubility or availability values of the phosphates.

It is evident that no relationship is found between the yields of mustard or ragi grains at harvest and solubility or availability values given in the third column of Table IV. In the case of ragi, however, some relationship is exhibited between its vegetative growth at early stage as detailed in the last column and the solubility values of the last five fertilizers. This is not of course exactly proportional; for, although the solubility values of these five manures are identical, the corresponding vegetative growth is not the same, but varies over a wide range. Even this relationship is not maintained at the time of harvest.

These results, however, indicate that solubility is not the dominating factor of availability of fertilizers in a soil. Biochemical activity of a soil, colloidal properties of the manures according to the hypothesis of Comber [1922], feeding power of plants, and base exchange phenomena along with other unexplored factors perhaps play an important role in rendering the phosphatic fertilizers available to crops grown in a particular

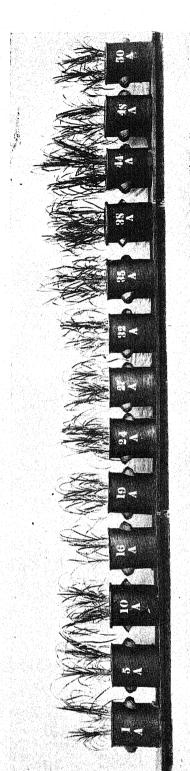


Fig. 1. Growth of ragi seedlings under various phosphatic fertilizers

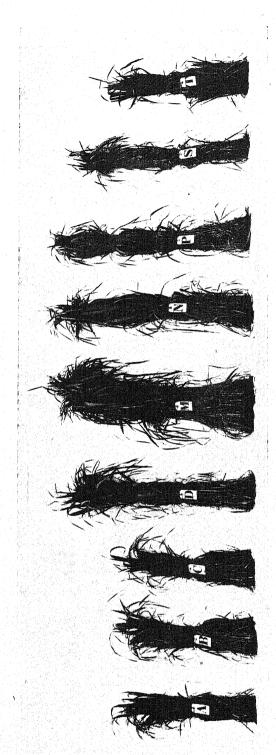


Fig. 2. Growth of oat seedlings in the field under various phosphatic fertilizers



South

Table IV
Relationship between solubility values of various manures and crop yields in a calcareous soil

Kind of phosphates applied	Per cent total	Per cent of total	Per cent v grain ove	ariation of er control	Per cent growth variation of ragi
	P.O.5	citric acid	Mustard	Ragi	seedlings over control
Iron phosphate Apatite Trichi-nodules Dicalcium phosphate Superphosphate Magnesium ammonium phosphate Aluminium phosphate Bonemeal Tricalcium phosphate Potassium phosphate Ammonium phosphate Ammonium phosphate	36·23 19·14 19·74 36·13 21·40 32·48 38·37 23·40 43·13 52·17 53·29	11·20 12·08 23·24 54·68 75·84 81·47 94·71 99·49 100·00 100·00	57·0 22·3 32·2 61·6 127·3 56·2 19·0 36·8 62·8 38·8 105·0	58·6 16·6 1·3 27·0 51·2 7·0 76·0 —20·9 69·4 62·5 36·6	-27-5 25-5 -29-4 40-2 86-3 -13-7 40-2 88-2 104-0 150-0 200-0

In order to test the conclusions with regard to the relative availability of different phosphatic fertilizers arrived at from pot experiments, field trials in the Punjab Experimental Area of the Pusa Farm were undertaken in the winter of 1934. Experiments were laid out in three adjacent 1/4 acre plots, each of which was subdivided into 18 equal sub-plots of 1/72 acre each measuring about 30 ft. north to south and 20 ft. east to west. A basal dressing of potash and nitrogen at 80 and 100 lb. per acre as sulphates of potassium and ammonium respectively was given to all the sub-

plots, allowance being made for the amount of nitrogen contained in the case of bonemeal and niciphos. Phosphatic fertilizers were applied 4 in below the surface by deep ploughing at 100 lb. of  $P_2O_5$  per acre according to the following plan based on controlled randomization of nine treatments of six replications each.

The treatments were control (C), apatite (A), bonemeal (B), disodium hydrogen phosphate (D), sodium metaphosphate (M), niciphos (N), sodium pyrophosphate (P), superphosphate (S) and Trichinodules (T).

Plan West

P	lot No.	29		Pl	ot No.	25	-	P	lot No.	21
SE	IAĻLO	w	DR.	AIN		SH	IALLOW		DRA	IN
<u>s</u>	P	T		. M	N	D		В	C	A
С	В	D		A	Р	s		T	М	N
N	A	S	AD	T	C	м	AD	P	D	В
T	С	P	ROAD	N	D	В	ROAD	A	S	M
M	N	В		S	A	P		D	T	C
A	D	M		В	T	C		N	P	S

North

East

Oat seeds were sown on 3 November 1934 in 30 lines east to west in each sub-plot at the rate of 40 lb. per acre. Owing to the paucity of germination there were gaps in certain lines, where seeds were resown. Twelve weeks after sowing, five representative plants were collected from each sub-plot. Thirty such plants thus taken from six sub-plots of each treatment were bundled together, weighed and photographed. (Plate III, fig. 2.)

The variation in vegetative growth as reflected in Plate III, fig. 2, is shown in Table V.

TABLE V

Fresh weight of oat plants of twelve weeks' growth in plots under different phosphatic fertilizers

Treatment	Fresh weight of 30 plants in gm.	Per cent variation over control
C = Control	890	 22·5
A = Apatite	$\frac{690}{720}$	-20·0
B = Bonemeal $D = Disodium hydrogen phosphate$	1,630	+83.2
M = Sodium metaphosphate .	2,900	+225.7
N = Niciphos · · ·	1,890	+112.4
P = Sodium pyrophosphate .	1,420	+60.0
S = Superphosphate	1,000	+12.4
T = Trichi-nodules	750	-15.7

It then became of interest to know how this variation in vegetative growth was reflected in the grain at harvest.

During harvest in March 1935, one row of plants from the surrounding border of each sub-plot was rejected. Mean yields of grain are given in Table VI along with their statistical examination according to Fisher's [1932] analysis of variance.

It is evident that the differences in mean yields of grain between the control and every other treatment are highly significant being greater than the critical value of difference even for one per cent level of significance, except in the case of indigenous phosphatic fertilizers, e.g. apatite, Trichi-nodules and bonemeal. Apatite and Trichi-nodules gave negative results and bonemeal hardly better yield, when compard with the control. Of the manures which have produced significantly better yields than the control, sodium metaphosphate heads the list with 31 per cent increase, and the rest give almost the same

Table VI

Mean yields of oats grain in field experiments with Pusa calcareous soil under various phosphatic fertilizers

Treatment	Mean yield in lb.	Per cent variation over control
C = Control A = Apatite B = Bonemeal D = Disodium hydrogen phos-	24·50 23·75 25·00 30·13	$-3.1 \\ +2.0 \\ +23.0$
$\begin{array}{lll} & & \text{phate} \\ M = & \text{Sodium metaphosphate} \\ N = & \text{Niciphos} \\ P = & \text{Sodium pyrophosphate} \\ S = & \text{Superphosphate} \\ \Gamma = & \text{Trichi-nodules} \\ \end{array}$	32·18 30·88 30·73 30·73 21·80	$+30.9 \\ +26.0 \\ +25.4 \\ +26.2 \\ -11.0$

Standard error for comparison of mean yields . . . . . = 2.075 Critical difference for 1 per cent . = 5.61 Critical difference for 5 per cent . = 4.19

increase ranging from 23 to 26 per cent over the control. In pot experiments, however, with mustard, the primary effect of Trichi-nodules and bonemeal on crop yield was significant but not the residual effect, whereas apatite gave no significant yield. In the present instance, metaphosphate gave the best vegetative growth and the indigenous phosphates less growth than even the control. This is fully reflected in the grain too. With regard to the rest of the phosphatic fertilizers, the differential vegetative growth at the early stage of the oat plants given in Table V is not reflected in the grain at harvest. For, all these phosphates give almost the same crop yield as shown in Table VI, which are, however, quite significant, when compared with the control.

It would thus appear from the cropping results of pot and field experiments that indigenous phosphates like bonemeal, apatite and Trichinodules proved very refractory in calcareous soils and were not rendered available to plants in field trials, whereas the last two became somewhat available in their primary effect in pot experiments with mustard, but their residual effect with ragi was not significant at all. Iron and aluminium phosphates which are normally taught to be unavailable to plants, proved quite useful as phosphatic fertilizers, the latter showing better residual effect than the primary. Sodium metaand pyro-phosphates, the two new products

prepared in the laboratory, show great promise as phosphatic fertilizers for calcareous soils. Metaphosphate proved the best of all the phosphatic fertilizers tried in the present experiments. The effect of the remaining phosphates both in pot and field experiments is quite appreciable.

#### SUMMARY AND GENERAL CONCLUSIONS

A series of pot experiments in a highly calcareous Pusa soil was conducted with indigenous phosphates like bones, apatite, and Trichi-nodules as well as artificial phosphates of iron, aluminium, calcium, magnesium, sodium, potassium and ammonium.

The cropping results with mustard showed 19 to 127 per cent higher yield of grain than the control according to the quality of phosphatic fertilizers used. The fertilizers could be classified into four broad groups according to the magnitude of yields obtained.

Eleusine coracana (vernacular ragi, marwa) was grown as the kharif (summer) crop in the same pots in order to study the residual effect of these manures. Iron and aluminium phosphates which are normally taught to be unavailable to plants,

proved quite useful, the latter showing better residual effect than the primary.

The examination of solubility or availability values of these manures by extraction with 2 per cent citric acid solution showed some relationship between them and crop growth of ragi at its early stage only with five of these manures, but such relationship was finally non-existent, when the respective crops were harvested. Solubility is not, therefore, the dominating factor of availability

of phosphatic fertilizers in a soil.

Field experiments with oats showed that indigenous phosphates like bonemeal, apatite and Trichi-nodules prove quite refractory in calcareous soils and sodium meta- and pyro-phosphates, newly used as phosphatic fertilizers, show great promise in calcareous soils. The metaphosphate proved the best of the remaining phosphates tried, e.g. niciphos, superphosphate, sodium phosphate and sodium pyrophosphate, all the latter giving almost identically satisfactory crop yields.

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## CERATOSTOMELLA DISEASES OF PINEAPPLE

By S. Chowdhury, Plant Pathological Laboratory, Sylhet, Assam

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(With Plate IV and five text-figures)

PINEAPPLE [Ananas comosus (L.) Merr.] (=A. sativus Lindl.) is grown extensively in the Surma Valley and the Hill Districts and its cultivation is extending to new land every year. Three diseases of this crop due to Ceratostomella paradoxa (de Seynes) Dade [=Thielaviopsis paradoxa (de Seynes) v. Höhn.] have been found widespread and occur throughout the pineapple growing tracts. These diseases are: leaf-spot, base-rot and fruit-rot. Careful survey and investigations made during the last four years have revealed that leaf-spot causes negligible damage, base-rot destroys 4 to 10 per cent of the plants and fruit-rot 3 to 15 per cent of the fruits. Fruit rot has been found to do more damage in storage and during transit than in the fields. The local

Joldhup variety has been found more susceptible to fruit-rot than the imported varieties, Giant Kew and Queen. During transit, in a few cases, Joldhup variety has been found to lose more than 50 per cent of the fruits.

Mehta [1940] reported a stem-end and soft-rot due to C. paradoxa in pineapples brought to the Cawnpore market from Gorakhpur in the United Provinces. There is no other mention of the occurrence of these diseases from any other part of India. In other countries these diseases have been noted from an early time. Larsen [1910] noted these in Hawaii, Patterson et al. [1910] in the United States of America, Roldan [ 1925 ] in the Philippine Islands, Dickson [1929] in Australia, Boedijn [1929] in Sumatra, Johnson

[1931] in the Antilles, Central America and Puerto Rico, Shepherd [1935] in Mauritius, Parham [1935] in Fiji, Bell [1936] in Queensland and Traub and Robinson [1938] in Mexico. Thus it is manifest that these diseases are quite wide-spread. Even in India, careful surveys are likely to show that they are prevalent in more localities than we know at present.

#### SYMPTOMS OF THE DISEASES

(i) Leaf-spot. The spots appear on any part of the leaf. They vary considerably as regards size, shape and colour. Many are large and white and are noticeable from a long distance while others are small and inconspicuous. In typical mature spots there is a straw coloured central area surrounded by a dark margin. Very often there is a dark center within the straw coloured area or dark blotches due to the formation of the black macrospores within the tissue. The internal tissue is soft and decayed at first but this soon dries out leaving the injured area dry and shrunken. In early stages the spots may be olive brown in colour and fairly regular in outline or they may be white and irregular from the start.

(ii) Base-rot. Pineapple suckers sometimes fail to develop normally after planting out. Such plants remain more or less at a standstill and cease to produce new growth. Later a yellowing and withering of the leaves commence and the sucker eventually dies. The plants exhibiting these symptoms will be found to be loose in the ground and closer examination will disclose black area of rot invading the base of the stem. The rot gradually extends until the whole of the lower part of the sucker together with some of the lower leaf bases may be involved. The plant is then liable to break off at the ground level. Older plants are also sometimes affected with this trouble, the symptoms being very similar to those described above.

(iii) Fruit-rot. The fruit tissue when affected takes on a water-soaked appearance, becomes a shade darker yellow than the normal tissue and has a characteristic odour. It is very soft and juicy even in the early stages of decay, and becomes so thoroughly disintegrated that it yields to the slightest pressure. Another and perhaps a more accurate means of distinguishing the rot is a black discolouration that takes place on the surface of diseased tissue when this has been exposed to the air. This black discolouration

consists of the dark coloured macrospores of the fungus. They develop so freely that the entire exposed surface becomes quite black in appearance. In advanced stages of the rot they may occur within the fruit especially along the core, but in most cases it is necessary to cut open the pineapple and expose the affected tissue to atmospheric conditions for about 24 hours before the black spores become apparent. In the final stages of rot, when the fruits are going to pieces, the whole fruit becomes covered with these spores. Plate IV shows the symptom of the disease.

#### PATHOGENECITY

The following inoculation experiments were carried out by the pure culture of the fungus:

(i) Leaves. A large number of leaves were selected. They were either wounded by sterile scalpels or left without wounds. The spores of the fungus were then either sprayed on their surfaces or bits of culture containing the mycelium and spores of the fungus placed at the wounded or other selected unwounded places. Leaves wounded before inoculation only took infection. On uninjured tissues the fungus grew under moist conditions but could not penetrate the leaf. It appears therefore that in the field, infection takes place through surface wounds made by insects and by the spines and sharp edges of the neighbouring leaves.

(ii) Base. Seventy crowns were inoculated with the fungus by injecting the fungus into the tissue at the base of the crown. Every one became affected with base-rot. When the fungus was applied over the surface of the tissue the plants were also affected. It was further demonstrated by experiments that infection may take place directly from the fungus that is present on the surface of the tissue at the time of planting. In the preliminary experiment successful infections were also obtained simply by inoculating the soil before planting. Therefore it is evident that the fungus present in the soil about the planting material may be responsible for infection. When the rot sets in before the cuttings are planted the infection takes place from fungus spores that happen to lodge towards the base of the planting material and which by virtue of moist conditions are induced to germinate.

(iii) Fruit. Both green and ripe truits were subjected to infection. Fruits to be inoculated were disinfected with 1:1000 mercuric chloride

solution and then washed thoroughly with sterile water. The fruits were then either sprayed with a spore suspension of the fungus or spores and hyphae of the fungus (bits of culture) applied at selected places on the wounded and unwounded surfaces of the fruits. The inoculated fruits were kept in moist chambers. From two to five days after inoculation the inoculated fruits showed the characteristic symptoms of the disease.

Inoculation experiments carried out on fruits have conclusively proved that the fungus is capable of infecting pineapple fruits with and without the help of wounds. It has further been brought out that the fungus can affect green pineapple as readily as the ripe one.

It was observed that when spore suspensions in sterile water were sprayed over the fruits in each case typical soft rot developed not from any single point in the surface as was the case when the fungus had been inoculated into a puncture but over the entire fruit at once affecting the tissue adjoining the surface and travelling gradually towards the centre. The point of infection in such cases seems to be in the crevices between the individual fruits or berries which make up the pineapple, for on examining the fruits infected by surface inoculation it was found that the rot had invariably proceeded from the bottom of these crevices into the surrounding tissue.

Such is probably the usual avenue of entrance followed by the fungus in attacking fresh fruits during transit or in storage. The transpiration from the fruits produces a moist atmosphere which readily permits spore germination and consequently surface inoculation takes place. The cut end of the stem also affords an avenue of entrance for the fungus under these conditions.

In the field much of the infection takes place through wounds in the fruit surface such as insect punctures, sun scald, fruit cracks and injuries inflicted by animals or implements during the various field operations. The dry atmospheric condition in the field prevents surface inoculation to a large extent. Only the very base of the fruit it seems affords such conditions as make surface infection in the field possible. At the base of the fruit a chamber is formed between the bracts on the stem and the base of the pineapple and the fungus spores which lodge here are protected from the sunlight and are often surrounded with sufficiently moist atmosphere to permit of their germination.

### CAUSAL ORGANISM

## (a) Growth in culture

The fungus was grown on a large number of media; potato-dextrose agar, oat meal agar, pineapple decoction, maize meal agar, pineapple fruit cylinders and steamed pineapple leaf. On all these substrata the fungus showed good growth. The cultural features of the fungus on all these various culture media did not differ greatly. The predominant snowy white growth of the cultures 24 hours old turning to grayish green, dark green and finally black as they became older was the conspicuous character of the fungus. The production of microconidia in cultures 24-48 hours old, followed later by the formation of macro-conidia was observed to take place in all cultures. The fungus showed such rapidity of growth in all cultures that the entire surface of the substrata was covered with the fungus growth after four days. The fungus however showed a tendency to produce less profuse growth on steamed pineapple leaf than on the other media.

## (b) Microscopic features

Mycelium. The mycelium of the fungus is hyaline, granular with a greenish tint frequently branched and septate. It becomes ragged and thick-walled with age. The breadth of the hyphae ranges from 2.5 to  $10.5\mu$ .

Conidiophores. The conidiophores are of two types (Figs. 1 & 2). One bears macro-conidia and the other bears micro-conidia. The conidiophores bearing macro-conidia are more or less club-shaped, septate, hyaline and sometimes granular. The number of septa varies from one to three. The length measures from 21.9 to 73.2  $\mu$ . They produce from 2 to 40 conidia in chains.

The conidiophores bearing micro-conidia are hyaline or may be granular tinged with light green colour. Their form is very characteristic. The basal portion is swollen but abruptly constricted at the point of attachment to the mycelium and tapers towards the apex presenting a candle-stick shape. The micro-conidiophores are septate. The septation varies from 2 to 13. The frequency of septation depends upon the length of conidiophores. The conidiophores vary from 34.9 to 250  $\mu$  in length. The micro-conidiophores produce two types of conidia which form chains of from 2 to 75.

Both the macro- and micro-conidiophores may be produced laterally from the same mycelium.

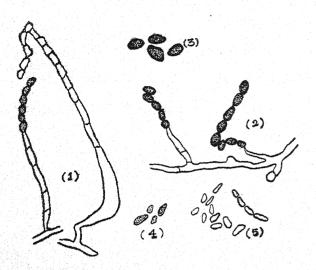


Fig. 1. Micro-conidiophores;

- 2. Macro-conidiophores;
- 3. Macro-conidia :
- Micro-conidia (light brown);
- 5. Micro-conidia (hyaline)

Macro-conidia. The macro-conidia (Fig. 3) are light to dark brown, smooth, ovoid or ellipsoidal, having thick walls. They are provided with strongly refractive contents and very often with one or more large vacuoles. The macro-conidia are pushed up from the conidiophores when new spores are produced below and finally form chains consisting of as many as 30 spores. The size of the macro-conidia ranges from 14-19×10-14 μ. On some media they form in such great profusion that they give the media an almost jet black appearance.

Micro-conidia. The micro-conidia are of two types (Figs. 4 & 5) and can be easily distinguished from each other on account of the sizes, form and colour. One is light brown and the other is hvaline. Both forms of micro-conidia are borne on the same kind of conidiophores.

The micro-conidia of light brown colour are barrel-shaped or ovoid, rarely cylindrical with thick wall varying from  $9.4-19\times4-6$   $\mu$ .

The hyaline micro-conidia are cylindrical with thin wall varying in size from  $9-12\times3\cdot5-5$   $\mu$ .

Both types of micro-conidia are formed in the same manner. They develop inside the hyphal cell of the conidiophores and are ejected one after

the other forming chains. The hyaline microconidia are produced more profusely and in chains of from 2 to 75 while the coloured micro-conidia are from 2 to 50 in a chain. Sometimes both forms of micro-conidia occur in the same chain interspersed promiscuously with one another.

## SPORE GERMINATION

Both forms of micro-conidia germinate after 5-8 hours in water or in any moist nutrient media. The germination of both forms of micro-conidia gives rise to the formation of hyaline germ tubes at the ends, a little below the ends or at the middle of the cell. In most cases the germ tubes developed readily into conidiophores producing micro-conidia in chains.

The macro-conidia germinate less readily than the micro-conidia; usually they germinate after 24 hours. They produce on germination hyaline germ tubes with swollen bases. The germ tubes were found to occur only at the middle of the cell and not to produce conidia immediately as the micro-conidia on germination.

#### PERPETUATION AND DISSEMINATION

The fungus is a facultative parasite and survives in the soil, on dead roots and crowns, leaf trash or ripe fruits of pineapple. Experiments conducted have shown that the fungus is killed by one hour's exposure to sunlight when grown on solid media but when grown on liquid media several hours' exposure is necessary. Under field conditions the fungus is not usually exposed to direct sunlight and as such it has practically no chance of being killed by being exposed to direct sunlight.

The fungus is disseminated by insects, wind,

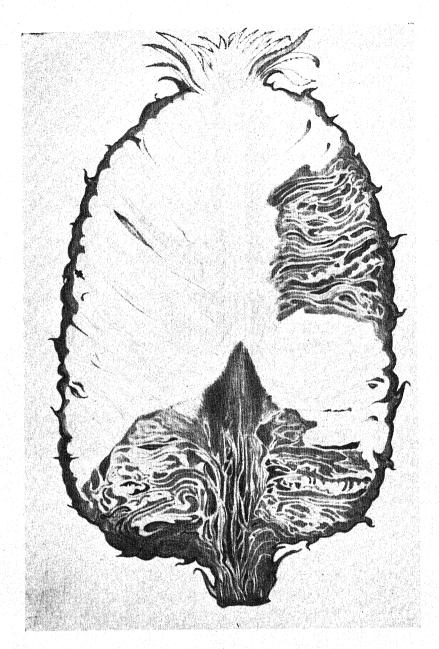
man and planting material.

#### SUMMARY

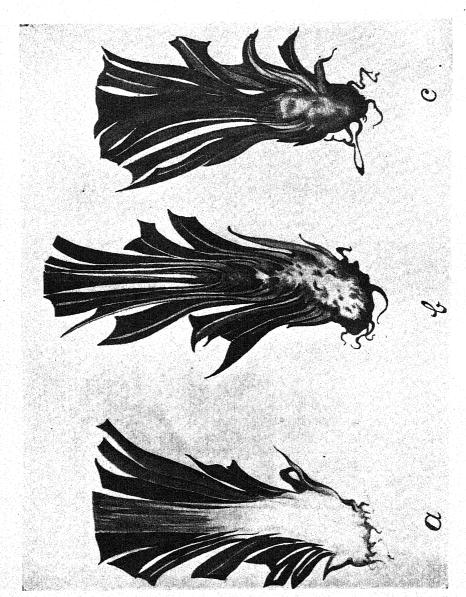
Three diseases of pineapple (Ananas comosus = A. sativus) due to Ceratostomella paradoxa (= Thielaviopsis paradoxa) have been noticed throughout the pineapple growing tracts in the Surma Valley and the Hill Districts. Thesediseases are: leaf-spot, base-rot and fruit-rot.

The symptoms of the diseases are described.

Inoculation experiments were carried out with pure cultures of the fungus and the pathogenecity established. It was found that the fungus could not infect unwounded leaves but both ripe and green fruits were equally susceptible to infection without the aid of wounds.



Symptoms of the disease: Thielaviopsis fruit rot of pineapple



Symptoms of the disease: Heart rot of young pineapple plantings— (a) Healthy; (b) and (c) diseased

The fungus was grown on a large number of media and its morphology studied.

Methods of spore germination and modes of perpetuation and dissemination were studied. It was found that the fungus survives in the soil, on dead roots and crowns, leaf trash and ripe fruits. The fungus is disseminated by insects, wind, man and through infected planting materials.

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## HEART OR STEM-ROT OF PINEAPPLE

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(With Plate V)

THROUGHOUT the pineapple [Ananas comosus (L.) Merr.] (= A. sativus Lindl.) growing tracts of the Surma Valley and the Hill Districts, young pineapple plants have been found susceptible to a destructive disease known as the heart or stemrot. Diseased plants die with the partial or complete destruction of the meristematic tissue of the stem. A careful survey made over the pineapple growing tracts during the last four years has revealed that the distribution of the disease in the different fields is highly sporadic, certain fields losing from 7 to 25 per cent of their plants and others losing none. It has also been noticed that the disease develops under damp water-logged conditions and is observed usually during the rains.

The disease has not so far been reported from any other part of India but it has been found quite prevalent in other pine growing countries. Henricksen [1905] noted this disease in Puerto Rico, Larsen [1910] in Hawaii, Ashby [1920] in Jamaica, Simmonds [1929] in Australia and Barnett [1931] in New South Wales.

## SYMPTOMS OF THE DISEASE

The disease is characterized in the early stages of infection by loss of turgidity and a slight twisting of the central leaves and in later stages

by the withering and discolouration to yellowish pink and brown. In both early and advanced stages the inner whorl of leaves can be readily detached from the stem by a slight pull, the basal tissues of all such leaves having undergone either a partial or complete disintegration. Natural falling of these leaves is a frequent late symptom. The stem may also show either a partial or complete disintegration with a characteristic brownish yellow discolouration at the margins between healthy and diseased tissues. The initial infection may appear at the apical end of the stem, at the base of some leaf, at the basal end of the stem or at the axillary or main roots. The infected tissues soon undergo decomposition. Plate V shows the symptoms of the disease.

#### IDENTIFICATION ofTHE PATHOGENE INFECTION EXPERIMENTS

A large number of isolations were made from diseased materials collected from the different pineapple growing tracts. A species of Phytophthora was always obtained in culture and all the isolates were found identical.

The morpholgical characteristics of the fungus were studied in culture and were found to agree with the description given for Phytophthora parasitica by Dastur [1913]. The fungus is therefore identified to be a strain of P. parasitica Dastur.

Infection experiments were carried out by placing bits of pure culture of the fungus at the base of the leaves, on the stem or on the roots of the host. Such infection experiments were repeated many times. Ninety-seven per cent of the inoculations were successful and the plants inoculated developed typical symptoms of the disease. Re-isolations were made and the fungus recovered in all cases. Controls kept remained healthy.

## CONTROL MEASURES

For control of heart or stem-rot providing the best drainage and avoiding wounding the planting material appear good precautions. In most cases heart rot attacks weak planting materials which should therefore be avoided. Mehrlich [1931] found that dipping the planting material in a very heavy Bordeaux (one pound copper sulphate crystals; one pound hydrated lime; three gallons of water) gave good control. This and a few other concentrations of Bordeaux mixture (2:2: 4:4:50 and 5:5:50) were given a wide trial by the author but the concentration suggested by Mehrlich has given the best results.

## SUMMARY

Heart or stem-rot of pineapple has been found to be a serious disease in the pineapple growing tracts of the Surma Valley and the Hill Districts.

The symptoms of the disease have been

described. The disease has been found due to Phytophthora

parasitica Dastur. Pathogenecity has been established by infection experiments.

The disease can be controlled by providing the best drainage, avoiding planting of weak materials and the dipping in of the planting material in a heavy Bordeaux mixture before planting.

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# CONTROL OF CERCOSPORA BLIGHT OF TIL

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(Received for publication on 14 December 1944)

T1L (Sesamum orientale L.) is grown extensively throughout Assam. Agricultural Statistics of Assam for the year 1942-43 report 342,906 acres under this crop in the province. A survey made during the last four years has shown that the only serious disease from which this crop suffers is a blight due to Cercospora sesami Zimm. and that this disease causes considerable monetary loss every year. Figures collected show that the yield is reduced 4.5 to 12 per cent due to this disease, in some cases the yield has been reduced even 21 per cent. It may safely be stated that on an average 5 per cent of the yield is reduced every year due to this disease. Taking 5 md. as the average yield of til per acre the loss would work out to be 85,741.5 md. per year or Rs. 8,57,410 per annum under the prevailing prices.

As the disease is a serious one a systematic study of the trouble was undertaken in 1940. A paper dealing with the physiology of the causal organism has been published by the author [1944]. In the present paper the methods of perpetuation. dissemination and control of the disease are presented.

## PERPETUATION OF THE DISEASE

(i) Seed transmission. A large number of seed samples collected from diseased crops were

examined and it was found that the fungus was present to a maximum extent of 27 per cent internally in seed samples. Seed saved from diseased crops were surface sterilized with 1:1000 mercuric chloride solution and then after careful washing with sterile water plated on oat agar. The fungus was obtained in culture in 50 per cent of the cases. Seeds saved from diseased crops were sown and diseased crops always obtained. From these experiments and observations it may be concluded that the fungus persists in the seeds. Doran and Guba [1928] obtained no evidence that Cercospora carotæ was carried on the seed. Klotz [1923], Lehman [1934], McKay and Pool [1918], Stewart [1910] and Wolf [1916] have cited evidence indicating that seed transmission occurs with species of Cercospora affecting celery, beets, dill, soybean and peanuts.

(ii) Persistence in the soil. Persistence of the fungus in the soil was investigated. Infected Sesamum leaves were collected in July 1941 and placed in wire containers. Some of these were placed on the surface of the soil, some 4 in. and some 6 in. deep. These containers were removed in May 1942 and typical Cercospora lesions were secured in plants inoculated with water suspensions of the decayed leaves. It is evident therefore that the parasite can persist in the plant debris left in the soil. Doran and Guba [1928] have reported that Cercospora carotae hibernates in dead leaves of carrots in or on the soil. Klotz [1923], Nagel [1938] and Pool and McKay [1916] found that other parasitic members of this genus over-winter on the dead leaves of their hosts.

From what has been stated above it will appear that the disease can be carried over through infected seeds or infected plant debris lying in the soil or both but experiments and observations made during the last four years have revealed that the disease is principally and primarily carried through infected seeds.

## DISSEMINATION IN THE FIELD

Observations indicate that wind is the probable agent of dissemination of the disease in the field. The prevailing wind is from the west and during harvest it has been noticed that the plants along the east side of a field are more severely blighted than those on the west. Sufficient inoculum apparently is carried by the wind to increase infection on the east side. Also when there are fields of young Sesamum on the east and west

sides of a field of older badly infected Sesamum, the former in the east field are more heavily infected. Agar plates were exposed for 3 min. at 10, 100 and 300 ft. distant from the leeward side of a field badly affected with Cercospora leaf blight. The fungus was recovered from the air at all three locations. Klotz [1923], Pool and McKay [1916], Roldan and Querijero [1939] and Wolf [1916] have reported that wind is an important agent in disseminating the species of Cercospora attacking celery, peanuts and beets.

#### CONTROL MEASURES -

Studies so far made have conclusively proved that the disease is principally and primarily carried through infected seeds. Several treatments were therefore applied to Sesamum seed to determine those that might be used without seed injury and would be effective in controlling the disease. Mercuric chloride (1:1000 for 5 min.) cuprous oxide dust, malachite green, copper sulphate, agrosan and formalin dusts were tried but none were found appreciably effective in controlling the disease. Hot water treatment as suggested by Nusbaum [1941] was therefore tried. Virtually complete control was effected by 30 minutes' immersion of the seed in water heated to 128°F. This was found effective in destroying the inoculum that was borne internally while surface borne inoculum was eliminated by treatment for the same period at 118°F. It was also found that one year's storage freed the seeds from superficial infection but the fungus still persisted in the interior. The practical utility of the hot water treatment was demonstrated on large scale plantings in 1943 and 1944.

#### SUMMARY

Cercospora blight of til (Sesamum orientale) due to Cercospora sesami is a serious disease in Assam. Figures collected show that the yield is reduced 4.5 to 12 per cent due to this disease; in some cases it is 21 per cent but the average comes to 5 per cent.

The disease is perpetuated through infected seeds and plant debris lying in the field.

In the field the disease is disseminated by wind. Seed treatments by chemicals have not been found effective in controlling the disease. Hot water treatment has given very satisfactory results on a field scale.

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## THE FUNGI OF BURMA

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(Received for publication on 12 January 1945)

THE fungus flora of Burma is rich and varied on account of the varied climatic conditions of the The first collection of Burmese fungi was made by Kurz, Curator of the Royal Botanic Gardens, Calcutta, about the year 1870 and a further collection was made by E. J. Butler, Imperial Mycologist, about the year 1912. Mycological work in Burma commenced in 1923 and the major portion of this work was devoted to the study of fungi responsible for the diseases of economic plants. During the course of tours, fungi found on wild plants, dead wood and the ground were also collected and added to the herbarium. The commoner fungi were examined and identified at the mycological laboratories at the Agricultural Research Institute, Mandalay, and the others were sent from time to time to the Imperial Mycological Institute, Kew, or the Imperial Mycologist, India, for identification. We are indebted to the various officers of these Institutions for their ready assistance.

At the time of the evacuation of Burma, in April 1942, the mycological herbarium consisted of over 700 specimens and of these about 200 still remained not fully named. Some of these unidentified fungi were no doubt of much systematic interest as some of these could not be placed in any of the known genera. For example, there was a specimen closely agreeing with Macrophoma but with the difference that there were clusters of hyaline appendages at each end of the spores.

The list given below does not pretend to be a complete record of all the fungi so far collected in Burma and undoubtedly the collection was very incomplete. The list has been composed partly from personal knowledge but mainly from various publications, the list of which is given. The loss of most of the laboratory records and herbarium material has been a serious hinderance but lest further information disappear this partial list is now published.

We are grateful to Dr B. B. Mundkur of the Imperial Agricultural Research Institute, New Delhi, for various suggestions in the preparation of this paper.

#### ARCHIMYCETES

Spongospora subterranea (Wallr.) Johns. On tubers of Solanum tuberosum, Hsipaw

#### PHYCOMYCETES

## **Oomyceles**

- Allomyces arbusculus Butler
- In soil from a pond, Rangoon
- Allomyces cystogenus Emerson
- In soil from a pond, Rangoon
- Allomyces javanicus Kniep
- In soil from a pond, Rangoon
- Cystopus bliti (Biv.) de Bary
  - On leaves of Amaranthus sp., Moulmein
- Cystopus candidus (Pers.) Lév.
- On leaves of Brassica campestris, Maymyo Cystopus ipomoeæ-panduratæ (Schw.) Stev. et
- Swing. On leaves of Ipomoeæ reinformis, Man lalay
- Cystopus portulacæ (DC.) Lév.
  - On leaves of Portulaca quadrifida, Bassein

Peronospora brassicæ Gäumann

On leaves and fruits of *Brassica campestris*, Amarapura

Peronospora effusa (Grev.) Rabenh.

On leaves of Chenopodium album, Myingyan Phytophthora colocasiæ Rac.

On leaves, petioles, flowers and corms of Colocasia antiquorum, Tavoy; in roots of Piper betle, Madaya

Phytophthora infestans (Mont.) de Barv

On leaves of Lycopersicum esculentum and Solanum tuberosum, Amarapura

Phytophthora palmivora Butler

On leaves, twigs, fruits and the tapping cut of *Hevea brasiliensis*, Thaton, Moulmein and Tavoy; on fruits of Carica papaya, Hmawbi

Plasmopara viticola Berk, and Curt.

On leaves of Vitis vinifera, Mandalay

Pseudoperonospora cubensis (Berk, and Curt.) Rost. On leaves of Lagenaria vulgaris, Amarapura Pythium aphanidermatum (Edson) Fitz.

On seedlings of *Nicotiana tabacum*, Mandalay; on rhizomes of *Zingiber officinale*, Moulmein

#### Zygomycetes

Choanephora cucurbitarum (Berk. and Rav.)
Thaxter

On flowers of Capsicum annuum, Mandalay Choanephora infundibulifera (Currey) Cunn.

On flowers of Hibiscus rosa-sinensis, Mandalay

Rhizopus artocarpi Rac.

On fruits of Artocarpus integer, Mandalay Rhizophus nigricans Ehrenberg
From soil, Mandalay

#### ASCOMYCETES

## Hemiascomycetes

Nematospora coryli Peglion

In unripe bolls of Gossypium neglectum and G. hirsutum, Mandalay; in young fruits of Abutilon indicum, Mandalay. The fungus follows the attack by the red cotton bug, Dysdercus cingulatus.

Nematospora gossypii Ashby and Nowell

In unripe bolls of Gossypium neglectum and G. hirsutum, Mandalay; in young fruits of Abutilon indicum, Hibiscus cannabinus, H. panduriformis and H. sabdariffa, Mandalay. The fungus follows the attack by the red cotton bug.

Nematospora sp.

In fruits of Citrus nobilis, Hsipaw

## Discomycetes

Humaria rutilans (Fr.) Sacc.

On mud banks, Toungoo

Tryblidiella rufula (Spreng.) Sacc.

On dead branches of Citrus sp. and Psidium guajava, Sagaing and Mandalay

Tuber indicum Cke and Massee In the earth, Mandalay

### Pyrenomycetes

Apiospora camptospora Penzig and Sacc.

On leaf-sheaths of Saccharum officinarum,
Bilin

Asterina lawsoniæ P. Henn et Nym.

On leaves of Lawsonia alba, Gyobingauk

Asterina magnifica Syd. et Butl.

On leaves of Terminalia sp., Moulmein

Balansia andropogonis Syd.

On inflorescence of Chrysopogon aciculatus,

Mandalay and Pa-an

Capnodium sp.

On leaves of Eryobotrya japonica, Maymyo; on leaves of Gossypium spp. and Mangifera indica, Mandalay; on leaves of Saccharum officinarum, Pyinmanna

Catacauma acaciæ Thiess. et Syd.

On leaves of Acacia leucophloea, Mandalay

Catacauma infectorium (Cke.) Thiess. et Syd.

On leaves of Ficus religiosa, Insein and Mandalay

Ceratostomella paradoxa (de Seynes) Dade

In fruits of Ananas sativa, Maymyo; on fruits of Areca catechu, Madaya; on trunk of Borassus flabellifer, Mandalay

Claviceps sp.

In ovaries of Sorghum dochna, Mandalay and Thaton

Clematomyces pinophili Thaxt.

On the inferior surface of Pinophilus sp., Pegu

Daldinia concentrica (Bolt.) Ces. et de Not.

On dead wood of Aleurites montana, Hsipaw Diplocarpon rosæ Wolf

On leaves of Rosa spp., Maymyo

Endodothella bambusæ (Rabenh.) Thiess and Syd,

On leaves of Bambusa sp., Moulmein Erysiphe cichoracearum DC.

On leaves of Cucurbita ovifera, Maymyo

Erysiphe polygoni DC.

On leaves and inflorescence of Coriandrum sativum, Mandalay

Glomerella gossypii (South.) Edg.

On bolls of Gossypium sp., Amherst

Hypocrella discoidea (Berk. and Br.) Sacc. On Aleyrodes on Tectona grandis, Rangoon

Hypocrella mollii Koorders

On Aleyrodes on Tectona grandis, Rangoon; on Aleyrodes on Castanopsis sp., Shan

Hypoxylon marginatum (Schw.) Berk.

On wood, Toungoo

Laboulbenia euschizomeri Speg.

On Euschizomerus æneus, Tikelæ

Laboulbenia crechtochili Thaxt.

On the margin of elytrae of Orechtochilus typus, Tenasserim

Laboulbenia crechtochilicola Speg.

On Orechtochilus feae, Tenasserim

Leptosphaeria sacchari Breda de Haan

On leaves of Saccharum officinarum, Pyinmanna and Bilin

Massarina usambarensis (P. Henn.) v. Hoehn.

On the bark of Citrus aurantium, Moulmein Meliola bicornis Wint.

On leaves on Desmodium sp., Basein

Meliola butleri Syd.

On leaves of Citrus decumana, Kya-in

Meliola cladotricha Lév.

On coriaceous leaves of an unknown host, Kya-in

Meliola jasminicola P. Henn.

On leaves of Jasminum sp., Gyobingauk

Meliola mangiferæ Earle

On leaves of Mangifera indica, Insein

Meliola pterospermi Stev.

On leaves of Pterospermum sp., Lower Burma Metasphaeria albescens Thuem.

On leaves of Oryza sativa, Hmawbi and Tavoy

Nectria bolbophylli P. Henn.

On grains of Oryza sativa, Hmawbi

Nectria diploa Berk. and Curt.

On a scale insect on Indigofera, Bassein

Nectria diversispora Petch

On fruits of Hevea brasiliensis, Mergui and Moulmein

Nectria eugniæ Currey

On dead leaves of Eugenia sp., Yomah

Nectria heterosperma Kalchbr. and Cke

On branches of Citrus aurantium, Sagaing

Neocosmospora vasinfecta Smith

On roots of Cicer arietinum, Padu

Peroneutypella pusilla Syd.

On dead branches of Citrus sp., Sagaing Phragmocapnias betle (Syd. et Butl.) Thiess. et

On leaves of Piper betle, Mudon

Phyllachora assimilis Syd.

On leaves of Capillipedium parviflorum, May-

Phyllachora centothecæ Syd.

On leaves of Centotheca lappacea, Moulmein

Phyllachora cynodontis (Sacc.) Niessl

On leaves of Cynodon dactylon, Mandalay

Phyllachora dalbergiæ Niessl

On leaves of Dalbergia lanceolata, Bassein

Phyllachora graminis (Pers.) Fckl.

On leaves of Pollinia grata (= Microstegium gratum), Moulmein

Phyllachora ischaemi Syd.

On leaves of Schima nervosum, Bilin

Phyllachora permixta Syd.

On leaves of Schima wallichii, Maymyo

Phyllachora rhytismoides (Cda.) Sacc.

On leaves of Mimosa sp., Tenasserim

Phyllachora Sacchari P. Henn.

On leaves of Saccharum officinarum, Pyinmanna

Phyllachora sacchari-spontanei Syd.

On leaves of Saccharum spontaneum, Bassein

Phyllachora sorghi v. Hoehn.

On leaves of Sorghum dochna and S. roxburghii, Tatkon

Phyllactinia corylea (Pers.) Karst.

On leaves of Morus sp., Mandalay and May-

Physalospora transversalis Syd.

On living leaves of Cocos nucifera, Bilin

Pleosphæropsis dalbergiæ Died.

On leaves of Dalbergia sissoo, Kyaukse

Pyrenocarpon magnificum (Syd. et Butl.) Theiss. On leaves of Terminalia sp., Moulmein

Rosellinia sublimbata (Durieu and Mont.) Pass. On stems of Thysanolæna procera, Kanbalataung

Sirrhodothis seriata Syd. and Butler

On leaves of Bambusa sp., Moulmein Sphaerostilbe repens Berk. and Br.

On roots of Hevea brasiliensis, Moulmein

Sphaerotheca humuli var. fuliginea (Schlecht.)

On leaves of Phaseolus aconitifolius, Mandalay

Uncinula tectonæ Salmon

On leaves of Tectona grandis, Lower Burma Ustilaginoidea ochracea P. Henn.

In the flowers of Panicum auritum, Hmawbi

Ustilaginoidea virens (Cke.) Tak.

In the flowers of *Oryza sativa*, Mandalay, Hmawbi, etc.

Ustulina zonata (Lév.) Sacc.

On trunk of *Hevea brasiliensis*, Moulmein and Tavov

Xylaria guyanensis Mont.

In ever-green forests, Toungoo

Xylaria hypoxylon (L.) Grev.

On old tree stumps, Arrakan

Xylaria nigripes (Klotzsch) Sacc.

On the ground and on termites nests, Mandalay

#### BASIDICMYCETES

#### Ustilaginales

Cintractia axicola (Berk.) Cornu

In the peduncles of Fimbristylis dichotoma, Mandalay

Entyloma eugeniarum Cke. and Massee [Petrak and Sydow (Ann. Mycol. 23: 261, 1925) state this is not a smut]

On leaves of Eugenia? tetragona, Maymyo

Entyloma oryzæ Syd.

On leaves of Oryza sativa, Hmawbi

Graphiola borassi Syd. and Butler

On leaves of Borassus flabellifer, Kyaukse Graphiola phoenicis (Moug.) Poiteau .

On leaves of *Phoenix* sp., Mandalay

Neovossia horrida (Tak.) Padwick and Azmat.

In ovaries of Oryza sativa, Mandalay and Hmawbi

Sorosporium furcatum Syd. and Butler

In ovaries of *Ischaemum aristatum*, Insein Sphacelotheca andropogonis-annulati (Bref.) Zundel.

In ovaries of *Dichanthium annulatum*, Mandalay

Sphacelotheca reiliana (Kuehn) Clinton

In inflorescence of Sorghum dochna and S. roxburghii, Tatkon

Sphacelotheca sorghi (Link) Clinton

In ovaries of Sorghum dochna and S. roxburghii, Mandalay

Ustilago burmanica Syd. and Butler In ovaries of Ischæmum sp., Kya-in Ustilago cynodontis P. Henn.

In inflorescence of Cynodon dactylon, Mandalay

Ustilago kolleri Wille

In ovaries of Avena sativa, Taunggyi

Ustilago scitaminea Syd.

In culms and terminal shoots of Saccharum officinarum, Pyinmanna

Ustilago tritici (Pers.) Rostr.

In ovaries of *Triticum astivum*, throughout Upper Burma

Ustilago utriculosa (Nees.) Tul.

In ovaries of Polygonum tomentosum, Rangoon

#### Uredinales

Aecidium mori Barclay

On leaves of Morus alba, Maymyo

Aecidium spissum Syd.

On leaves of Zanthoxylum sp., Maymyo Cerotelium desmium (Berk. and Br.) Arth.

On leaves of Gossypium sp., Allanmyo

Cerotelium fici (Cast.) Arth.

On leaves of *Ficus carica*, Mandalay; on leaves of *F. palmata*, Mandalay and Maymyo

Hapalophragmium ponderosum Syd. and Butler On twigs of Acacia leucophloea, Mandalay

Hemileia vastatrix Berk. and Br.

On leaves of Coffea arabica, Leiktho

Masseeella fluggeæ Syd.

On leaves of Fluggea microcarpa, Padu

Phakopsora phyllanthi Diet.

On leaves of Phyllanthus disticus, Mandalay

Phakopsora zizyphi-vulgaris (P. Henn) Diet.

On leaves of Zizyphus jujuba, Mandalay

Phragmidium burmanicum Syd.

On leaves of Rubus lasiocarpus, Maymyo Sydow transfers this to the new genus Phragmotelium.

Puccinia arundinellæ Barclay

On leaves of Arundinella sp., Maymyo

Puccinia burmanica Syd. and Butler

On leaves of Anthistrria imberbis (=Themeda triandra), Maymyo

Puccinia butleri Syd.

On leaves and stalks of Launaa asplenifolia Amarapura

Puccinia glumarum (Schm.) Erikss. and Henn.

On leaves, culms and glumes of *Triticum* astivum, Mandalay

Puccinia graminis Pers.

On leaves, culms and glumes of Triticum astivum and Hordeum vulgare, Mandalay

Puccinia inayati Syd.

On leaves of Launæa nudicaulis, Lower Burma

Puccinia kozukensis Diet.

On leaves of Andropogon micranthus (= Capillipedium parviflorum), Maymyo

Puccinia kuehnii (Krueg.) Butler

On leaves of Saccharum spontaneum, Bassein

Puccinia prainiana Barclay

On leaves of Smilax sp., Bilin

Puccinia purpurea Cke

On leaves of Sorghum dochna and S. roxburghii, Mandalay; on leaves of Sorghum halepene, Mandalay

Puccinia romagnoliana Maire and Sacc. On leaves of Cyperus sp., Hmawbi

Puccinia rufipes Diet.

On leaves of Imperata cylindrica, Moulmein

Puccinia solmsii P. Henn.

On leaves of Polygonum sp., Maymyo

Puccinia sorghi Schw.

On leaves of Zea mays, Amarapura

Puccinia thwaitesii Berk.

On leaves of Justicia gendarussa, throughout Burma

Puccinia triticina Erikss.

On leaves of Triticum æstivum, throughout Upper Burma

Puccinia versicolor Diet and Holw.

On leaves of Heteropogon contortus, Maymyo

Pucciniastrum castaneæ Diet.

On leaves of Castanopsis javanica, Maymyo

Ravenelia emblicæ Syd.

On leaves of Phyllanthus emblica, Maymyo

Sphærophragmium acaciæ (Cke.) P. Magn. On leaves of Albizzia lebbeck, Myitnge

Tranzschelia pruni-spinosæ (Pers.) Arthur On leaves of Prunus armeniaca and P. persica,

Maymyo

Uredo cajani Syd

On leaves of Cajanus cajan, Tatkon

Uredo fuirenæ P. Henn.

On leaves of Fuirena glomerata, Bassein

Uredo tectonæ Racib.

On leaves of Tectona grandis, Madaya

Uromyces appendiculatus (Pers.) Link

On leaves of Dolichos lablab and Vigna unquiculata, Amarapura

Uromyces commelinæ Čke

On leaves of Commelina bengalensis, Mandalay

Uromyces decoratus Syd.

On leaves and stem of Crotalaria juncea, Amarapura

Uromyces fabæ (Pers.) de Bary

On leaves of Pisum arvense and P. sativum,

Maymyo

Uromyces linearis Berk and Br.

On leaves of Panicum repens, Mandalay

Uromyces mucunæ Rabenh.

On leaves of Mucuna sp., Maymyo

## Hymenomycetes

Amanitopsis sp.

On the ground, Kanbalu

Auricularia mesenterica Fr.

On decaying wood, Mandalay

Collybia albuminosa (Berk.) Petch

On termites nests, Tonbo

Coprinus spp.

On dung and paddy straw heaps, Mandalay

Corticum salmonicolar Berk. and Br.

On branches of Hevea brasiliensis, Mergui and Thaton; on branches of Cinchona sp., Mergui

Corticum spp.

On branches of Aleurites montana, Hsum-Hsai; on decomposing paddy straw, Mandalay

Dædalea discolor Fr.

On the ground, Pegu Yoma

Daedalea tenuis Berk.

On dead wood, Pegu Yoma

Daedalea unicolar (Bull.) Fr.

On stumps, Pegu

Daedalea zonata Schwein.

On dead wood, Toungoo

Entoloma microcarpum Berk, and Br.

On termites nests, Tonbo

Exobasidium vexans Massee

On leaves of Camellia sinensis, Toungoo Hills

Fomes holosclerus Berk.

On dead wood, Pegu Yoma

Fomes lamaoensis (Murr.) Sacc. and Trott.

On roots of Broussonetia papyrifera and Hevea brasiliensis, Thaton

Fomes marginatus Fr.

On dead trees, Toungoo Hills

Fomes pseudoferreus Wakefield

On roots of Hevea brasiliensis, Moulmein

Galera zeylanica Petch

On the ground, Mandalay

Ganoderma applanatum (Pers.) Pat.

On tree trunks, Toungoo

Ganoderma lucidum (Leyss.) Karst.

On trunk of Areca catechu, Madaya, causing the stem-bleeding disease; on Morus sp., Maymyo

Guepinia spathularia (Schw.) Fr.

On old logs, Mandalay

Hexagonia similis Berk.

On dead wood of Mangifera indica, Mandalay

Hirneola auricula-judæ (L.) Berk.

On dead wood and fallen branches, Kanbalu

Hirneola nigra (Swartz) Fr. On logs, North Yoma

Irpex pallescens Fr.

On old wood, Pegu Yoma

Lentinus capronatus Fr.

On dead wood, Myodwine

Lentinus curreyanus Sacc. and Cub.

On dead wood, Lower Burma

Lentinus descendens Fr.

On dead wood, Toungoo

Lentinus polychrous Lév.

On dead wood, Yoma Range

Lentinus velutinus Fr.

On dead wood, Pegu Yoma

Lentinus sp.

On dead shoots of Bambusa sp., Mandalay Edible

Lenzites albida Fr.

On dead wood, Toungoo

Lenzites repanda (Mont.) Fr.

On dead tree trunks, Toungoo

Lepiota sp.

On the ground amongst grass, Mandalay. Edible

Marasmius burmensis Cke.

On twigs, Moulmein

Marasmius sacchari Wakker

On exotic varieties of Saccharum officinarum, Mandalay

Naucoria sp.

On the ground, Mandalay

Panaeolus cyanescens Berk. and Br.

On cowdung heaps, Mandalay. Poisonous

On dead wood, Mandalay. Edible

Polyporus bicolor Jungh.

On wood, Toungoo

Polyporus rhodophaeus Lév.

On dead wood, Toungoo

Polystictus cinerescens Schwein.

On wood, Pegu Yoma

Polystictus flabelliformis Klotzsch.

On dead wood, Yoma Range

Polystictus modestus Kunze

On dead wood, Yoma Range

Psathyrella sp.

On the ground, Mandalay

Schizophyllum commune Fr.

On dry fruits of Garcinia mangostana, Mudon, and on dead wood throughout Burma

Stereum adustum Lév.

On wood, Southern Yoma

Stereum elegans Meyer

On wood, Lower Burma

Stereum lobatum Fr.

On dead wood, Toungoo

Stereum princeps Jungh.

On dead wood, Toungoo Hills

Trametes cingulata Berk.

On dead wood, Pegu Yoma

Tremella sp.

On dead wood, Mandalay

Volvaria diplasia Berk. and Br.

On rotten paddy-straw heaps, all Burma. Edible

Gasteromycetes

Bovista brasiliensis (Fr.) de Toni

On the grounp, Toungoo

Dictyophora? indusiata (Ventenat) Pers.

On ground in rubber estate, Moulmein

Geaster sp.

On the ground, Kanbalu. Edible

Ithyphallus impudicus (L.) Fr.

On the ground, Mandalay

Lycoperdon sp.

On the ground, Madaya

Podaxon pistillaris (L.) Fr.

On rich sandy soils, Madaya. Edible

Fungi imperfecti

Hyphomycetes (and Mycelia sterilia)

Actinonema rosæ (Lib.) Fr.

On leaves of Rosa sp., Maymyo

Alternaria brassicæ (Berk.) Sacc.

On leaves of Brassica campestris, Amarapura

Alternaria circinans (Berk. and Curt.) Bolle

On leaves of Brassica oleracea, Mandalav

Alternaria solani (Ell. and Mart.) Jones and

Grout

On leaves of Solanum tuberosum, Amarapura

Aspergillus flavus Link

In isolations from paddy soil, Mandalay

Aspergillus fumigatus Fresenius On match sticks, Mandalay

Aspergillus niger van Tieghem

In isolations from paddy soil, Mandalay; on scales of Allium sp., Mandalay

Aspergillus tamarii Kita

In isolations from paddy soil, Mandalay

Aspergillus terreus Thom

In isolations from paddy soil, Mandalay

Cercospora coffeicola Berk. and Cke

On leaves of Coffea arabica, Leiktho

Cercospora dolichi Ell. and Ev.

On leaves of Dolichos lablab, Amarapura

Cercospora feuilleauboisii Sacc.

On leaves of Solanum melongena, Mandalay

Cercospora henningsii Allesch.

On leaves of Manihot utilissima, Mudon

Cercospora Hibisci Tracy and Earle

On leaves of Hibiscus sabdariffa and H. cannabinus, Mandalay

Cercospora kopkei Krug.

On leaves of Saccharum officinarum, Sahmaw

Cercospora menispermi Ell. and Holw.

On leaves of Cocculus vilosus, Mandalay

Cercospora neriella Sacc.

On leaves of Nerium odorum, Mandalay

Cercospora nicotianæ Ell. and Ev.

On leaves of Nicotiana tabacum, Amarapura

Cercospora oryzæ Miyake

On leaves of Oryza sativa, all over Burma

Cercospora patouillardi Sacc. and D. Sacc. On leaves of Calotropis gigantea, Mandalay

Cercospora personata (Berk. and Curt). Ell. and E. On leaves of Arachis hypogaea, all over

Burma

Cercospora sesami Zimm.

On leaves of Sesamum orientale, Tatkon

Cercospora sorghi Ell. and Ev.

On leaves of Sorghum dochna and S. roxburghii Mandalay

Cercospora traversiana Sacc.

On leaves of Trigonella fænu-graecum, Mandalay

Cercospora viticola (Ces.) Sacc.

On leaves of Vitis vinifera, Insein

Cercospora sp.

On leaves of Mimosa pudica, Hmawbi

Cerebella andropogonis-contorti Subram.

On ovaries of Heteropogon contortus, Maymyo This and other species of Cerebella grow

upon forms of Sphacelia on the various hosts

Cerebella burmanensis Subram.

Man-On ovaries of Brachiaria setigera, dalay

Cerebella cynodontis Syd.

On ovaries of Cynodon dactylon and Brachiaria reptans, Mandalay

Cerebella inquinans (Berk. and Br.) Petch

On ovaries of paspalum scrobiculatum, Bassein and Hmawbi

Cerebella volkensii (P. Henn.) Mundkur

On ovaries of Sorghum dochna, all over Upper Burma

Cladosporium herbarum (Pers.) Link

On grains of Triticum aestivum, Mandalay; on capsules of Sesamum orientale, Tatkon

Cladosporium zizyphi Karst. and Roum.

On leaves of Zizyphus jujuba, Mandalay Coniosporium bambusæ (Thum. and Bolle) Sacc.

On culms of Bambusa sp., Insein

Curvularia lunata (Wakker) Boedijn

In isolations from paddy soil, Mandalay; on flowers of Dichanthium, annulatum, Mandalav

Exosporium palmivorum Sacc.

On leaves of Borassus flabellifer, Moulmein

Fusarium moniliforme Sheld.

Causing top-rot disease of Saccharum officinarum, Pyinmanna

Fusarium udum Butler

On roots and stem of Cajanus cajan, Tatkon

Fusarium vasinfectum Atk.

On roots of Gossypium neglectum, Mahlaing Fusarium sp.

On stem and capsules of Sesamum orientale, all over Burma

Helminthosporium cynodontis Marignoni

On leaves of Cynodon dactylon, Mandalay

Helminthosporium gramineum Rabenh.

On leaves of Hordeum vulgare, Amarapura Helminthosporium oryzæ Breda de Haan.

On leaves and grains of Oryza sativa, all over Burma

Helminthosporium sativum Pam., King and Bakke On leaves of Triticum æstivum, all over Upper Burma

Helminthosporium teres Sacc.

On leaves of Hordeum vulgare, Amarapura

Helminthosporium turcicum Pass.

On leaves of Zea mays, Amarapura

Helminthosporium sp.

On leaves, stem and capsules of Sesamum orientale, Allanmyo

Macrosporium parasiticum Thuem.

On leaves of Allium sp., Myitha

Metarrhizium anisopliæ Sor.

On grubs of Oryctes rhinoceros, Madaya

Nigrospora sphaerica (Sacc.) Mason

On leaves of Oryza sativa, all over Burma

Oidiopsis taurica (Lév.) Salmon

On leaves of *Tropæolum majus*, Maymyo; on leaves of *Solanum melongena*, Amarapura; on leaves of *Capsicum annuum*, Mandalay

Oidium erysiphoides Fries

On Pisum sativum, Maymyo; on Sesamum orientale, Tatkon, on Lagenaria vulgaris, Amarapura; on Vigna unguiculata, Amarapura; on leaves and pods of Cajanus cajan, Taunggyi; on Cucurbita spp., Amarapura

Oidium mangiferæ Berthet

On inflorescence of Mangifera indica, Mandalay

Penicillium digitatum Sacc.

On fruits of Citrus aurantium, Mandalay

Penicillium italicum Wehm.

On fruits of Citrus aurantium, Mandalay

Piricularia grisea (Cke.) Sacc.

On leaves of Digitaria pruriens, Amarapura

Piricularia oryzæ Cavara

On leaves of Oryza sativa, throughout Burma

Ramularia areola Atkinson

On leaves of Gossypium neglectum, Tatkon

Rhizoctonia solani Kühn.

In roots of Cicer arietinum, Gossypium sp., Nicotiana tabacum, Phaseolus acutifolius, P. lunatus, P. vulgaris and Solanum tuberosum, throughout Upper Burma

Sphacelia sorghi McRæ

On inflorescence of Sorghum dochna, Mandalay and Tatkon

Sphacelia spp.

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On dead fruits of Psidium guajava, Mandalay

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Aschersonia badia Patouill.

On (insects on) living leaves of bamboo, Pegu Yoma Aschersonia cinnabarina P. Henn.

On leaves of Tectona grandis, Lower Burma

Ascochyta citri Penzig

On leaves of Citrus decumana and C. medica, Maymyo

Botryodiplodia theobromæ Patouill.

On culms of Saccharum officinarum, Pyinmanna; on stem of Hevea brasiliensis, Mergui; on terminal twigs of Ceiba pentandra (Kapôk), Kyaukse; on roots of Manihot qlaziovii, Myaungmya

Colletotrichum camelliæ Massee

On leaves of Camellia sinensis, Leiktho Colletotrichum capsici (Syd.) Butl. and Bisby

On stem and fruits of Capsicum annum, Mandalay

Colletotrichum coffeanum Noack

On leaves of Coffea arabica, Leiktho

Colletotrichum falcatum Went

On leaves and culms of Saccharum officinarum, Pyinmanna

Colletritchum glæosporides Penz.

On leaves and stems of various Citrus spp., Shan States

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On leaves of Sorghum spp., throughout Burma

Colletotrichum lindemuthianum (Sacc. and Mag.) Br. and Cav.

On pods of Dolichos lablab, Amarapura

Colletotrichum nigrum Ell. and Hals.

On fruits of Capsicum annuum, Myitha

Collectotrichum spp.

On leaves of Mangifera indica, throughout Burma; on leaves of Themeda sp., Tonbo

Diplodia natalensis Pole Evans

On fruits of Garcinia mangostana, Mudon; on fruits of Mangifera indica, causing stemend rot, throughout Burma; on fruits of Citrus medica and C. nobilis, throughout Shan States

Diplozythiella bambusina Died.

On leaves of Bambusa sp., Madaya

Dothiorella ribes (Fuckel) Sacc.

On fruits of *Mangifera indica*, causing stemend rot, throughout Burma

Glæosporium elasticæ Cke and Mass.

On leaves of Ficus elastica, Maymyo

Glæosporium musarum Cke. and Mass.

On over-ripe fruits of Musa sapientum, throughout Burma

Glæosporium piperatum Ell. and Ev.

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Pestalotia psidii Pat.

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On leaves of Terminalia catappa, Maymyo

Phyllosticta religiosa Syd.

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Marasmius sacchari

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Claviceps sp.

Collectotrichum graminicolum

Phyllachora sorghi Puccinia purpurea Sphacelotheca reiliana

Sphacelotheca sorghi

Sterculia alata Roxb.

Glæosporium sp.

Tectona grandis L. f. Aschersonia cinnabarina

Hypocrella discoidea

Hypocrella mollii

Phyllosticta tectonæ

Uncinula tectonæ

Uredo iectonæ

Terminalia catappa L.

Glæosporium terminaliæ

 $Phyllosticta\ catappx$ 

Terminalia sp.

Asterina magnifica

Pyrenocarpon magnificum

Themeda (Anthistiria) triandra Forsk.

Puccinia burmanica

Themeda sp.

Collectotrichum sp.

Thysanolæna procera Mez.

 $Rosellinia\ sublimbata$ 

 $Trigonella\ fœnum-graceum\ {\bf L}.$ 

Cercospora traversiana

Triticum æstivum L.

Cladosporium herbarum

Helminthosporium sativum

Puccinia glumarum

Puccinia graminis

Puccinia triticina

Sclerotium rolfsii

Ustilago tritici

Tropæolum majus L.

Oidiæopsis taurica

Vigna unguiculata (L.) Walp.

Oidium erysiphoides

Uromyces appendiculatus

Vitis vinifera L.

Cercospora viticola

Plasmopara viticola

Zanthoxylum sp.

Aecidium spissum

Zea mays L.

Diplodia sp.

Helminthosporium turcicum

Puccinia sorghi

Zingiber officinale Rosc.

Pythium aphanidermatum

Zizyphus jujuba Lamk. Cladosporium zizyphi Phakopsora zizuphivulgaris

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## INDIAN PLANTS LIABLE TO PRODUCE DERMATITIS

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THERE are a number of plants which are capable of producing irritation of the skin. This may be brought about by contact with the skin as in the case of some species belonging to the genera Rhus, Holigarna, Urtica, etc. resulting in minor or temporary irritation of the skin or painful irritation and inflammation with vesicles or blisters, depending on the severity of the contact and the susceptibility of the individual. Further, there are certain plants, such as Fagopyrum esculentum, Moench., Hypericum perforatum, Linn. etc. which if ingested by certain livestock under certain conditions lead to a photosensitization and consequent dermatitis of the unpigmented portions of the skin. Dermatitis may, therefore, be either produced by contact with irritant substances produced by the plant or by ingestion of the plant itself. An important point to remember in the case of these plants is that a number of them produce dermatitis only occasionally in individuals who are especially susceptible to them. Others are more troublesome and cause dermatitis in many, but not all, individuals who may come in contact with them or eat them.

The spines and thorns of a number of plants are also capable of entering the skin and setting up irritation. In some cases when the punctures so formed in the skin become subsequently infected with harmful micro-organisms serious septic wounds may be produced. Such plants are found in abundance in India, but obviously the injury they inflict is mechanical and hence they cannot come under the category of poisonous plants. They are, therefore, excluded from the scope of the present paper. On the other hand, the hair on the pods of some species of Mucuna have more or less a mechanical action in producing irritation; these have been included in this paper. The reason is that unlike the sharp spines which

produce merely mechanical injury they produce prolonged irritation and itching due possibly to the presence of certain chemical substances. It has been considered desirable, therefore, to draw attention to the existence of such plants the irritation produced by which very closely resembles that produced by stinging nettles.

In India approximately 76 plants occur which are capable of producing dermatitis in susceptible individuals. In some cases their action is explicable by the presence of irritant substances produced by the plants, in other cases the phenomenon is not yet fully understood. The following are some of the important types of plants which may injure the skin and which are usually met with in this country:

(1) Rhus type where the juice of plant comes in contact with the skin and produces dermatitis: Sollmann [1936] writing about some of the foreign species of Rhus says "Contact with certain species of Rhus common along roadsides, on fences, in woods and swamps, etc., produces typical dermatitis passing through the successive stages of hyperemia and itching, to voilent vesication, edema, and suppuration, according to the specific sensibility of the individual; many persons are practically immune, although a sufficient quantity of the isolated toxicodendrol has never failed to produce dermatitis.

The active ingredient of all the species is a phenolic oily resin, toxicodendrol, contained in the sticky sap of the plants, which exudes when the plant is injured. It is identical or very similar in all the toxic Rhus species. It is so highly active that 1/1,000 mg. has caused severe vesication. Toxicodendrol is not volatile, but it may be conveyed to some distance in the soot in the smoke of burning plants, and perhaps on dust, and by insects alighting on injured plants. None is

present in the pollen, as has been claimed. It may be conveyed by the hands or clothing from one person to another, as if it were contagious." Travellers in the Himalayan forests often hear some of the villagers having almost similar belief regarding the Indian species of Rhus. They would not touch the rhus trees or have anything to do with them; some of them actually avoid even passing under them. Even the smoke, smell or sight, they say, will cause swelling and vesication of the skin. And yet it has been observed that many individuals are immune to these plants. To a lesser extent species of Holigarna are similarly dreaded in India. Such cases of poisoning may be treated thus: After contact the exposed part may be freely washed with some alkaline solution. A 5 per cent solution of ferric chloride or ordinary soap solution are best used for the purpose. Before exposure, use of this measure may prevent the manifestation of harmful effects. Local application of baking soda or Epsom salts, one or two teaspoonful to a cup of water, or a 5 per cent solution of potassium permanganate may relieve the pain caused by inflammation. Fluid extracts of Grindelia, diluted with 6 to 10 parts of water is recommended for preventing the spread of inflammation. Ointments containing fatty or oily substances should not be used as the poison is soluble in oils and will, therefore, spread over other parts. Such emollients may, however, be applied as soothing agents, after the poison has been thoroughly washed away. It has been found by experiment that a certain amount of tolerance to the toxic effects of this plant may be developed in man by giving per os small and increasing doses of an alcoholic extract made from the plant to susceptible individual. Attacks of dermatitis in man caused by these plants may be prevented by subcutaneous injection of the alcoholic extract. The immunity produced by this method, however, does not persist longer than one month [Schamberg, 1919].

Important families which include plants whose juices are harmful are Anacardiaceae, Asclepiadaceae, Araceae and Euphorbiaceae. Species of Semecarpus, Rhus, Holigarna, Excoecaria, Euphorbia, Calotropis, Arisaema, etc. are the well-known

examples in the point.

(2) Urtica type where apparently the stiff hairs on the plant are responsible for producing dermatitis: Urticaria produced by contact with the

hairs on the stinging nettles, such as species of Urtica, Girardinia, Laportea, Fleurya, Tragia, etc. is well known. This urticaria is an inflammatory disorder accompanied by a considerable burning and itching in the affected part. The rash may come out in large or small patches, remaining for a few minutes or several hours and may disappear quite abruptly. It usually leaves no trace behind. Laportea crenulata Gaud. is perhaps the worst of all stinging nettles found in India. Contact with its hairs produces severe burning pain which may last for several days and is said to be greatly aggravated by the application of water. The sting is particularly powerful during the flowering season when it is said to bring on voilent sneezing, sleeplessness and fever, hence the local English names (Fever nettle, Devil nettle) by which the plant is known to coffee planters. According to Haines [1921-25] the plant is quite innoccuous at some times of the year. This may be so on account of the hairs being deciduous, and that they are especially abundant on the inflorescence, but we have never found the plant to be entirely harmless at any time. Haines remarks that while cutting coupe-lines in November in the Sikkim Tarai, where it is sometimes gregarious, his coolies were attacked with sneezing, violent catarrh and ultimately vertigo, apparently from inhaling the numerous minute hairs. Out of all these stinging nettles the mechanism of producing dermatitis in the case of Urtica dioica Linn. is well understood, and it is likely that others may resemble this plant to a greater or less degree. What happens in the case of Urtica dioica is that the very fragile ends of the hair penetrate the skin and are broken off. The irritating principle from inside the hair is brought in contact with the tissues and the uncomfortable itchy sensation accompanied by nettle rash supervenes. It has generally been accepted that the irritating material in the stinging hairs of this plant is formic acid, but investigation by Cleery [1927] has thrown a considerable light on the subject. According to this author the protoplasm of these hairs has an alkaline reaction, and encloses an acid cell sap. The cell sap contains a small amount of formic acid as well as acetic, butyric, and other volatile fatty acids. The specific poison of the cells, which is a non-volatile substance of an acid nature allied to the resin acids, is in solution in these acids. It is neither formic acid, nor probably an enzyme, or a toxalbumin.

According to Cleery [1927] it is without doubt allied to the irritant substances found in some Primulaceae, Anacardiaceae and allied plants.

A popular remedy against the stings of these stinging nettles is to rub over the affected part the leaves of Rumex nepalensis Spreng. and R. orientalis Bernh. which are commonly met with and are often found near the nettles. They afford substantial relief, but if these are not available one's own saliva rubbed in is quite effective. Dilute ammonia is a good remedy and if available should be rubbed in over the affected parts.

(3) Photosensitization caused by the ingestion of plants: Some plants, such as Fagopyrum esculentum Moench. and Hypericum perforatum Linn., if ingested under certain conditions and stages of growth are capable of producing photosensitization and consequent dermatitis of unpigmented portions of the skin. All kinds of stock and laboratory animals which have an unpigmented skin and which are exposed to sunlight after the ingestion of the plant are liable to get this condition. Animals having pigmented skins or those not exposed to bright sunlight do not develop any symptoms. Photosensitization can be prevented by (a) feeding these plants to stabled animals only and discontinuing the feeding about a month before animals are sent out to graze; (b) allowing such animals to graze in the early morning, late afternoon and at night only; and (c) by covering or staining albinos and white parts of pigmented animals. When the animal is actually affected, feeding must be discontinued at once. The animal must be immediately shaded and a purgative given. Symptomatic treatment should also be applied. Among human beings, certain individuals are known to be sensitive to buckwheat which comes under this category. Severe itching is experienced and a rash is produced after eating food made from buckwheat flour.

(4) Miscellaneous: Some plants, such as

Xanthium strumarium Linn., produce dermatitis only in very few individuals who are sensitive to this plant and that too only at the pre-fruiting stage. This has been observed in the case of our own plant collector who has often suffered by contact with this plant. The poisonous principle responsible for this action is not known. In other cases, such as in Lasiosiphon eriocephalus Decne., contact with the plant under natural conditions does not usually produce dermatitis. The dust from dried plants produces smarting of the eyes and mucous membranes and even of the intact skin.

Essential oils contained in the plants are sometimes responsible for irritating the skin, such as in the case of *Erigeron canadensis* Linn. and *Ruta graveolens* Linn. The resin from the rootstocks of *Podophyllum hexandrum* Royle is capable of producing severe irritation of the eyes and the mucous membranes generally. There are in addition a number of plants the exact mechanism of whose action or the active principles responsible for producing dermatitis are not yet fully understood. Research could be profitably undertaken on these plants so that rational treatments may be evolved against their injurious effects.

Below is given a list of plants occurring in India, which have been responsible for producing dermatitis. Their English names, common vernacular names, distribution, part or parts responsible for this condition and their chemical constituents and other general information, so far as these are known, are indicated for each species.

We are grateful to Col. Sir Ram Nath Chopra, C.I.E., for the valuable help and advice he has given us.

The following are the abbreviations used for the vernacular names:

Be.—Bengali; Bo.—Bombay; Hi.—Hindi; Kash.— Kashmir; Kum.—Kumaon; Ladd.—Laddakh; Lep.— Lepcha; Mal.—Malayalam; Mar.—Marathi; Nep.— Nepali; Pers.—Persian; Pun.—Punjabi; Sans.— Sanskrit; Tam.—Tamil; Tel.—Telugu.

Name of plant	Distribution	Constituents	The part or parts of the plant which cause dermatitis
1. Abroma augusta Linn, f. English— Devil's Cotton Vernacular— Hi. & Be.—Ulatkambal; Bo.—Ulaktambal	A shrub, native or cultivated, throughout the hotter parts of India from the North-West India to Sikkim at alt. of 3,000 ft.; Khasia mountains at altitudes of 4,000 ft., and Assam.		Irritant hairs

Name of plant	Distribution	Constituents	The part or parts of the plant which cause dermatitis
2. Ailanthus altissima (Mill.) Swingle (Syn. A. Glandulosa Desf.) English— Ailanto, Chinese sumach, Japan varnish tree, Stinking cedar, Tree of the Gods, Tree of heaven	A large deciduous tree met with in the hills of Northern India, most probably intro- duced from Japan.	The flowers contain an essential oil [Isaev, 1932]. The bark contains 0-005 per cent of a very bitter crystalline substance named allanthin and probably also a glucoside and a saponin [Wasicky and Orien, 1933].	Leaves, flowers
3. Anacardium occidentale Linn. English— Cashew nut, Cashew apple Vernacular— Hi. & Bo.—Kaju; Be.— Hijli badam	A small tree originally introduced from South America; now established in the coastal districts of South India, Chittagong, and the Andaman Islands.	The cellular pericarp is full of a black, caustic, only juice which is a powerful rubefacient and vesicant. It contains the phenolic compound cardol, anacardic acid and an ether-soluble substance to which cantharidin-like effects of the oil are attributed [Joseph and Sudborough, 1923].	Juice from the pericarp and trunk is very caustic and produces blisters. The nut within which is the kernel (the cashew nut) must be roasted to get rid off the poisonous substance. The fumes arising from their roasting are very irritating.
4. Anagallis arvensis Linn. English— Bird's eye, Red chickweed, Shepherd's calendar, Shepherd's delight Vernacular— Hi.—Jonkhmari	An erect or procumbent annual found over the greater part of India ascending to an altitude of 8,000 ft. in the Himalayas. The red-flowered variety is found in Kashmir, but the blue-flowered one is more common in India.	The herb [Wehmer, 1929-31; Supplement, 1935] contains two glucosidic saponins while the root contains cyclamin which is also a glucoside.	Leaves
5. Anthemis colula Linn	A foetid-smelling, acrid, erect annual herb found in Baluchistan and probably in Sind also.	The fresh plant yields 0.01 per cent and fresh flowers about 0.013 per cent of an essential oil [Hurd, 1885; Haake, 1891].	Leaves and flowers
6. Apium graveolens Linn. (wild form) English— Celery, Marsh parsley Vernacular— Hi. & Sans.—Ajmoda; Be.—Chanu	A biennial herb found at the foot of the North-West Himalayas and outlying hills in the Punjab. It is cultivated in different parts of India during the cold weather, chiefly as a garden crop in the vicinity of towns for use as a salad and as a potherb. Sometimes it is cultivated in Bengal for its seed and in the Punjab for its roots.	2.0 to 3 per cent.	According to Watt [1889-96] and Pammell [1911] the plant is irritant.  Muenscher [1939] states that leaves are irritant.
7. Arisaema speciosum (Wall.) Mart. Vernacular— Pun.—Kiralu, Samp-ki- khumb	A herb found in the temperate Himalayas, from Hazara to Sikkim and Bhutan at altitudes of 7,000 to 10,000 ft.		Juice, especially from tubers

Name of plant	Distribution	Constituents	The part or parts of the plant which cause dermatitis
8. Arisaema tortuosum (Wall.) Schott. Vernacular— Pun.—Don, Kiri-ki- Kukri	A tall plant found in the temperate and subtropical Himalayas from Simla to Bhutan at altitudes of about 8,000 ft., also in Khasia Hills, Manipur, Chota Nagpur, Ranchi and Parasnath. In western India it is found in Konkan, and in the Madras Presidency in Rampa Hills at 4,500 ft., Horsleykonda at 4,000 ft., and in Western Ghats at 3,000 to 7,000 ft. above sea level. According to Duthie [1903-29] the Dehra Dun plant which is found at altitudes of 3,000 ft. differs considerably from the typical A. tortuosum of Schott.		Juice, especially from tubers
9. Asparagus officinalis Linn. English— Asparagus, Sparrow grass Vernacular— Hi.—Halyun; Be.— Hikuna			Young stems [Muenscher, 1939]
10. Calotropis gigantea (Linn.) Dryand. Vernacular— Hi.—Ak; Sans.—Arka; Be. & Bo.—Akanda	A stout shrub frequently met with throughout India as a weed on fallow land and in waste ground except in the Punjab where it is sometimes found in gardens only (Fl. Brit. Ind. mentions the plant as occurring in the Punjab, but this seems doubtful to us).	The fibre of this plant contains a toxic bitter substance. The juice contains a proteolytic enzyme similar to papin [Basu and Nath, 1933; 1936]. The roots contain a gutta-percha-like substance (madar alban) consisting of isovaleric esters of two alcohols, and also a bitter yellow resinous substance [Sharma, 1934; Hill and Sarkar, 1915].	Milky juice. Its action is particularly severe on mucous membrane.
11. Calotropis procera (Linn.). Dryand. Vernacular— Hi.—Ak, Madar, Mundar; Sans.—Alarka; Bo.—Mandara	A shrub found more or less throughout India in warm and dry places from the North-West Frontier Province and in the Punjab to Western, Central and Southern India, it occurs abundantly in Sub-Himalayan tracts and the adjacent plains in the North-West.	The milky juice contains a proteolytic enzyme and a toxic substance [Gerber, and Flourens, 1913]. It also contains a highly active resin [Gerber and Flourens, 1912]. The root bark contains a bitter yellow resin but no alkaloid [Sharma, 1934].	Milky juice. A child about three years old, accidentally during play, brought the juice in contact with his prepuce. He was brought to the hospital two days afterwards. The part was very much swollen and there were patches of ulceration with narcosis. The patient had difficulty in passing urine. On circumcision the glans penis was also found to be swollen and ulcerated.

	Name of plant	Distribution	Constituents	The part or parts of the plant which cause dermatitis
12.	Cannabis sativa Linn. English— Hemp. Vernacular— Hi., Be. & Bo.—Bhang, Ganji; Sans.— Ganjika	This plant is found in many parts of India on waste ground and by the roadside. In the Himalayas it grows wild and is widely distributed, while it is acclimatized in the plains and is also occasionally cultivated.	The resinous matter (charas) found on the leaves, young twigs, bark of the stem and unfertilized flowers of the female plant contains about 1.5 per cent of a terpene, about 1.75 per cent of a sesquiterpene, a hydrocarbon monakosane and a toxic red oil, which is the active constituent of the plant. The approximate yield of the red oil is 33 per cent of the resin [Wood, Spivy and Easterfield, 1896; Marshal, 1897].	Leaves, flowers
13.	Cissus setosa Roxb (Syn. Vitis setosa Wall.) English— Hairy wild vine Vernacular— Hi.—Harmel; Khajgoli-chavel	A shrub found in Western India from the Circars and Mysore southwards		Juice
14.	Datura stramonium Linn. English— Devil's apple, Jimson weed, Thorn apple Vernacular— Pun.—Tettu dutura; Bo.—Sada dhutura	A coarse, annual herb found on the Himalayas from Kashmir to Sikkim up to an altitude of 9,000 ft. It is also met with in the hilly tracts of central and southern India.	The content of the alkaloid in the leaves varies from 0·3 to 0·5 per cent, the chief alkaloid being hyoseyamine. The bark, wood and pith of the stems contain 0·25, 0·09 and 0·20 per cent of the alkaloids respectively. The roots contain 0·1 to 0·25 per cent of alkaloids and the seeds 0·46 to 0·52 per cent [Feldhaus, 1905]. In some Indian specimens the total alkaloids in stems and roots vary from 0·19 to 0·26 per cent and in leaves and fruits from 0·38 to 0·45 per cent [Andrews, 1911].	Leaves, flowers, fruits
15.	Daucus carota Linn. English— Carrot Vernacular— Hi., Bo. & Pun.—Gojar; Sans.—Shikha-mulam	A hispid biennial herb cultivated throughout India as an article of food	The fruit of the cultivated carrot yields 1 to 1.5 per cent of an essential oil containing l-α- pinene, and a crystalline body named daucol [Finnemore, 1926]. The leaves contain the two bases pyrrolidine and daucine [Pictet and Court, 1907] besides an essential oil.	It has been stated that some persons develop dermatitis on coming in contact with carrot leaves, especially when they are wet.
16.	Delphinium ajacis Linn	Cultivated in gardens .	The seeds contain 2 alkaloids crystalline ajacin and crystalline ajaconin [Wehmer, 1929-31, Supplement 1935].	Leaves, seeds

	Name of plant	Distribution	Constituents	The part or parts of the plant which cause dermatitis
17.	Dictamnus albus Linn. English— Bastard dittany, Fra- xinella, White dittany	A strong-smelling shrubby plant, met with on the temperate Western Himalayas from Kashmir to Kunawar at altitudes of 6,000-8,000 ft.; very common in Pangi.	The roots contain an ethereal oil, a bitter substance, a saponin, a crystalline dictamnolaction (probably identical with evodin), a crystalline toxic alkaloid dictamnin and also a phenolic substance. The flowers contain 0.05 per cent and leaves 0.15 per cent of an essential oil [Wehmer, 1929-31, Supplement 1935].	Leaves, capsules
18.	Erigeron canadensis Linn. English— Cobbler's pegs, Canada fleabane Vernacular— Kash.—Kach	An annual herb found in the Western Himalayas, Punjab and Rohilkhund up to an altitude of 4,000 ft. It is plentiful in certain valleys in Kashmir. It is also found in Shillong (Assam) and on the Western Ghats and Nilgiris up to 6,000 ft. above sea level.	Fresh leaves contain 0.33 to 0.66 per cent and dry leaves 0.26 per cent of an essential oil [Wehmer, 1929-31, Supplement 1935; Rabak, 1905]. Tannic acid and gallic acid have also been isolated from the leaf [Wehmer, 1929-31, Supplement 1935].	Leaves. The oil has an acrid taste and causes smarting of the eyes and soreness of the throat. The leaves produce irritation of the parts of the body coming in contact with the plant,. When powdered the leaves produce a dust which is irritating [Pammell, 1911].
19	. Euphorbia acaulis Roxb. (Syn. E. fusiformis Buch Ham.)	Found in the tropical Hima- layas, Oudh, Bengal and western India		Milky juice very acrid and vesicant
20	). Euphorbia antiquorum Linn Vernacular— Hi.—Tridhara-sehund; Sans.—Vojrakantaka; Be.—Tekuta sij; Bo.—Narasej i	A large shrub or a small tree found in dry places throughout the hotter parts of India ascending to an altitude of 2,000 ft. It is also occasionally cultivated as hedge plant in villages.		Milky juice intensely irritant. During the collection of juice by the authors, the person employed for the purpose complained bitterly of itching all over the face, which was also considerably swollen. The trouble, however, was relieved by the application of a soothing preparation for a couple of days.
2	<ol> <li>Euphorbia cattimando W. Elliot (E. trigona Fl. Brit Ind., in part)</li> </ol>	An erect shrub found on the dry rocky hills in the Deccan, and probably other parts of India	Contains euphorbon [Henke, 1886].	Milky juice, which is vesicant in fresh condition
- 2:	2. Euphorbia helioscopia Linn. English— Cat's milk, Sun spurge Wartwort Vernacular— Hi.—Hirruseeah; Pun. Ganda bute	spring throughout the plains of the Punjab and the Siwalik tract, ascending	also an acid saponin which is strongly [haemolytic [Gonnerman, 1919]. The seeds contain 32.6 per cent of a fatty oil the physiolo- gical action of which is due	the application of a poultice made from the bruised plant.

	Name of plant	Distribution	Constituents	The part or parts of the plant which cause dermatitis
23. E	uphorbia neriifolia Linn.  Vernacular—  Hi.—Sehund; Sans.—  Snuhi; Be.—Massu-  s j; Bo.—Minyuta	A large fleshy shrub occasionally planted in villages as a hedge plant throughout India and is sometimes found wild on waste land. In Orissa and in the Deccan it is said to occur in a state of nature in rocky places.		Milky juice is rubefacient and acrid
	Euphorbia nivulea Buch Ham. Vernacular— Be.—Sij; Bo.—Newrang Sans.—Patta k u ir	A large shrub or a small tree found in dry rocky places in Northern, Central and Southern India		Milky juice
25. 1	Euphorbia peplus Linn.	Probably an introduced species	The herb contains 4.8 per cent of an oleo-resin [Vevey, 1908]. It also contains neutral and acid saponins with haemolytic properties [Gonnerman, 1919].	Milky juice
26. 1	Euphorbia rothiana Spreng.	Found in Central, Western and Southern India		Milky juice
27.	Euphorbia royleana Boiss. Vernacular— Hi. & Pub.—Shakar pitam, Thor	A fleshy shrub common on the dry and hot rocky slopes of the outer ranges of the Western Himalayas from the Indus to Kumaon, ascending to an altitude of 6,000 ft.; also on the Salt Range in the Punjab. It is also commonly grown in hedges in the Sub-Himalayan tract and the adjacent plains.		Milky juice. Very injurious to the eyes.
28.	Euphorbia thomsoniana Boiss. Vernacular— Kash.—Hirtiz	It occurs in Western Tibet, Kurrum, Kashmir (Gilgit), etc. at altitudes of 10,000 to 12,000 ft.		Milky juice
29.	Euphorbia tirucalli Linn.  English—Milk bush, Indian tree spurge  Vernacular—Hi.—Sehund Be.—Lanka sij; Bo.—Shera	An unarmed shrub or a small tree which is a native of Africa and has become naturalized in several places in India. It is often grown as a hedge or occasionally as a road-side tree.	[Wehmer, 1929-31, Supplement 1935].	Milky juice is rubefacient and vesicant. It produces severe inflammation and excruciating pain if it gets into a cut in the skin or into the eyes. It is said to be used by criminals to destroy the eyes of domestic animals.
30.	Euphorbia trigona Haw. (E. trigona Fl. Brit. Ind., in part)  Vernacular—Tel.—Kattinandu	An erect, glabrous shrub found in the dry rocky hills in the Deccan.	The milky juice contains euphorbon, resin, rubberlike substances and malic acid [Wehmer, 1929-31. Supplement 1935].	in fresh condition is vesicant.

Name of plant	Distribution ,	Constituents	The part or parts of the plant which cause dermatitis
31. Excoecaria agallocha Linn.  Fnglish—Blinding tree  Vernacular—Be.—Gangwa;  Bo.—Geva	A small tree found in tidal forests and swamps on all the coasts of India.		The fresh sap is extremely acrid and causes intolerable pain if it accidentally gets into the eyes, which sometimes happens to wood cutters when the tree is cut for fuel; hence the name 'Excecaria'. It blisters the skin and produces sores.
32. Fagopyrum esculentum Moench. English—Buckwheat, Brank Vernacular—Hi.—Kou, Kaltu, Phaphra; Pun.—Darau, Phaphar, Obal; Kash.—Trumba shirin.	An annual herb extensively cultivated in the Himalayas and Sub-Himalayan tracts and in Western Tibet at altitudes of 2,000 to 12,000 ft.; also in the Khasia Hills, Manipur, as well as in the hilly districts of Central and Southern India.	The herb contains the glucoside rutin, the seeds contain a substance which is toxic to lower animals  Ohmke, 1909]. The roots are said to contain oxymethylanthraquinones [Maurin 1925; 1926].	All parts of the plant, whether dry or fresh, are capable of producing photosensitization (fagopyrism) in animals, the fresh plant in the flowering stage being considered most toxic. The symptoms are: inflammatory swelling accompanied by severe itching of the ears, face and eyelids, spreading on to the sub-maxillary region and neck. In severe cases vesicles containing yellowish fluid appear on the affected part. These vesicles may become infected with bacteria and give rise to purulent and even narcotic dermatitis. Among human beings certain individuals are known to be sensitive to buckwheat. They experience severe itching and develop a rash from eating food made from buckwheat flour.
33. Fleurya interrupta Gaudich. Vernacular—Hi.—Lal bichua	An erect herb found in Bihar, Central Bengal and Khasia. Hills. In the Bombay Presidency it is met with in Konkan, Southern Mahratta Country and Kanara. In the Presidency of Madras it has been reported from the hills south of Mysore at altitudes of 5,000 to 6,000 ft. above sea level and also from the Rampa hills of the Eastern Ghats.		Stinging hairs on plant
34. Ginkyo biloba Linn. English— Maiden-hair tree	Rarely cultivated in gardens. There is one tree in Amritsar.	The leaves contain five crystalline substances and also shikiminic acid. The seeds contain gingkol acid, two alcohols, ginnol and bilobol, and also asparagin [Webmer, 1929-31, Supplement 1935].	

Name of plant	Distribution	Constituents	The part or parts of the plant which cause dermatitis
35. Girardinia heterophylla Decne. The varieties zeylanica and palmata of the Flora of British India are now considered by several botanists as distinct species, viz. G. zeylanica Decne. and G. leschenaultiana Decne.	A perennial herb found in the subtropical and temperate Himalayas from Kashmir to Sikkim, up to 7,000 ft. above sea level, also in Assam and the Khasia Hills.	•••	Stinging hairs on the plants
respectively. G. zeylanica is found in the southwestern hilly portion of the United Provinces and extends through Chota Nagpur, Mt. Abu, Konkan and the Deccan to the hills of Southern India and the west coast of Madras Presidency from 1,000 to 5,000 ft. above sea level. G. leschenaultiana is mor restricted in its distribution and is found on the mountains of the Wester Ghats at altitudes of 4,000 to 7,000 ft. Both these are known as Nilgiri nettles while the name Himalayan nettle is restricted to G. heterophylla.  English—  Himalayan nettle Vernacular—  Hi.—Alla, Bichua, Chichr; Pun.—Anjan, Bhabar, Karla			
36. Hedera helix Linn. English— Barren ivy, Creeping ivy, Ivy Vernacular— Hi.—Lablab; Pun.— Banda; Kash.—	Khasia Hills from 4,000 to 6,000 ft.	Nearly all parts of the plant, viz. leaves, fruits and seeds contain the glucoside α-hederin and probably certain other glucosides [Van der Haar, 1912; 1913; Block, 1888].	Leaves
Karmora 37. Hippomane mancinelle Linn. Fnglish—	duced from America, it is now occasionally cultivated	It contains acrid milky juice [Wehmer .1929-31, Supplement 1935].	
$egin{array}{ll} egin{array}{ll} egin{array}{ll} egin{array}{ll} egin{array}{ll} egin{array}{ll} egin{array}{ll} Arnottian \\ Hook f. \\ Vernacular & BoBibu \end{array}$	in Indian gardens. A tall tree found in the evergreen forests on the Western Ghats from the Konkan southwards.	•••	Juice. In some persons it produces blisters, while others are immune. The tree is dreaded by the local people.
39. H. grahamii (Wight) Hook	A tree found in Western India		The juice has properties similar to those of H arnottiana.
Vernacular— Mar.—B ilwuli, Bipte 40. H. longifolia BuchHan ex Roxb. Vernacular— Be.—Barola, Bo.—Hul	Deligar and Chicongong.		The juice is of a powerfully caustic nature and blisters the skin.

Name of plant	Distribution	Constituents	The part or parts of the plant which cause dermatitis
41. Humulus lupulus Linn.	It is cultivated in the North- West Himalayas	The active principles consti- tute the lupulin	Leaves
English— Hops.  42. Hypericum perforatum Linn. English— St. John's grass, St. John's wort Vernacular— Hi. and Pun.—B s- sant	A perennial herb found in the Western temperate Himalayas from Kumaon between 6,000 to 9,000 ft. to Kashmir between 3,000 to 6,500 ft. above sea level.	The herb contains tannins and 0.065 per cent of an essential oil [Zellner and Porodko, 1925].	Several investigators have reported that the plant in the flowering stage, if eaten in large amounts by livestock, leads to a photosensitization and consequent dermatitis of the unpigmented portions of the skin. Animals having pigmented skins or those not exposed to bright sunlight do not develop any symptoms, but white-skinned horses, cattle and sheep
43. Læportea crenulata Gaudich. English— Devil nettle, Elephant nettle, Fever nettle Vernacular— Hi.—Utijun; Be.— Chorpatta	Himalayas from Sikkim eastward, also in Assam and the Khasia Hills. In the	papers consulted by them.	develop characteristic symptoms (see under Fagopyrum esculentum) if exposed to sunlight. The toxic substance, it appears, acts upon the nerve-endings so as to photosensitize them and if the animal is sub- sequently exposed to strong sunlight, it develops dermatitis, including blistering of the skin and falling off of the hair. Stinging hairs on plant. It is perhaps the worst of all the stinging nettles found in India. A

	Name of plant	Distribution	Constituents	The part or parts of the plant which cause dermatitis
44.	Laporteu terminalis Wight	An erect herb found in the subtropical Himalayas from Kumaon to Mishmi at altitudes of 4,000 to 8,000 ft., in the Central Provinces at altitudes of 4,000 to 6,000 ft., and in the evergreen forests of the Western Ghats of the Madras Presidency at altitudes of 5,000 to 7,000 ft. It is also found in the Nilgiris.		Stinging hairs on plant
45.	Lasiosiphon eriocephalus Decne. English— Woolly-headed gnidia Vernacular— Bo.—Rumetha	A shrub, sometimes a small tree, found in the open forests of the Western Ghats of the Bombay and Madras Presidencies ascending to an altitude of 7,000 ft. in the Nilgiris.		The bark (and perhaps the leaves also) is powerfully vesicant. The collector of the bark for examination by the authors complained bitterly of a burning sensation in the eyes, nostrils and face during packing of the dried bark in bags. This sensation lasted, more or less, for three days.
46.	Leonurus cardiaca Linn.	A herb found in temperate Western Himalayas from Kashmir to Kumaon at altitudes of 6,000 to 10,000 ft.	The herb contains an amorphous bitter substance leonurin [Wehmer, 1929-31, Supplement 1935].	Leaves
47.	Lobelia excelsa Lesch.	A herb which grows on the Western Ghats of South India, the Nilgiris, Pulney Hills and hills of Travancore at altitudes of over 5,000 ft.	····	Milky juice
48.	Lobelia nicotianifolia Heyne English— Wild tobacco Vernacular— Hi.—Nala, Narasala; Be.—Balanala, Nala; Bo.—Bokenal, Dhavala	A herb found on the Western Ghats from Bombay to Travancore at altitudes of 3,000 to 7,000 ft. above sea level and is met with in Konkan, the Deccan, the Nilgiris, Malabar, etc.	The leaves contain two alkaloids one of which resembles lobeline from L. inflata Linn. [Dragendorff and Rosen, 1886].	Milky juice. The dust from the powdered herb irri- tates the nostrils in the same way as tobacco.
49.	Mucuna atropurpurea DC.	A woody climber found in the plains of Western India		See Mucuna prurita
50.	Mucuna gigantea DC. English— Elephant cowitch Vernacular— Mal.—Kakavalli; Tam.—Kalgaivalli; Tel.—Enugadulagondi	A woody climber found in the plains of Western India.		See Mucuna prurila

Name of plant	Distribution	Constituents	The part or parts of the plant which cause dermatitis
cuna hirsula Wight & A	n annual climber found in the plains of Western India	••••	See Mucuna prurita
cuna monosperma DC. glish— Negro bean rnacular— Bo.—Sonogravi, Mothi kuhili	woody climber of the Eastern Himalayas and Khasia Bills, also met with in Assam, Chittagong, and the hills of Western India.		See Mucuna pr <b>u</b> rita
cuna prurita Hook.  M. pruriens Fl. Brit. ind., non DC.) glish— Cowhage, Cowitch rnacular— Hi.—Kiwach; Sans.— Atmugupta; Be.—	an annual climber found in the Himalayas and the plains.		The rigid, pointed hairs on the pods, if touched enters the skin and produce itching. The action ap- pears to be purely mecha- nical.
Alkushi; Bo.—Kuhili			
rium oleander Linn.	Rarely cultivated in gardens; a plant of the Mediterranean region.	The leaves contain the gluco- sides neriin and oleandrin	Leaves
dophyllum hexandrum Royal (Syn. P. emodi Wall. ex Hook f. & Thoms.) nglish— Ducks foot, May apple ernacular— Banwangan; Pun.—Bankakri	A herb found in the interior ranges of the Himalayas at altitudes of 9,000 to 14,000 ft. from Sikkim to Hazara descending to 6,000 ft. in Kashmir.	The rootstock yields resin (podophyllin) and crystallised podophyllotoxin 10 per cent and 3.5 per cent respectively.	Rootstocks. Podophyllin greatly irritates the eyes and the mucous membranes generally. The collectors of this drug have to be very careful.
lygonum hydropiper Linn. nglish— Biting pepper, Smart- weed srnacular— Be.—Packur-mul	A herb found in wet places more or less throughout India, ascending to an altitude of 7,000 ft. in the Himalayas.	The herb contains formic acid, acetic acid and baldrianic acid, much tannin and small amounts of an essential oil [Steenhauer, 1919]. The root is said to contain oxymethyl-anthraquinones [Maurin, 1925; 1926].	The fresh plant contains an acrid juice which causes irritation and smarting when brought into contact with the nostrils or eyes. The bruised leaves as well as the seeds will raise blisters if employed as a poultice, as in the case of mustard poultice.
nunculus sceleratus Linn. nglish— Marsh crowfoot, Water celery ernacular— Kum.—Shim; Pers.— Ki b'kaj	An erect, annual herb met with on river banks in Bengal and Northern India, in the marshes of Peshawar and in the warm valleys of the Himalayas. It appears during the cold weather and remains until the break of the rains.		Leaves. The fresh plant is highly acrid. The bruised leaves when applied to the skin raise blisters and were formerly used in Europe by professional beggars to produce or maintain blisters or open sores to excite sympathy.
eus insignis Hook f. ernacular— Lep.—Serh; Nep.— Kagphulai	A small tree found in the interior valleys of the Sikkim Himalayas at altitudes of 3,000 to 6,000 ft., and in the Khasia Hills at 4,000 ft.		Leaves, bark, fruit. The juice is a powerful vesicant.
Marsh crowfoot, Water celery ernacular— Kum.—Shim; Pers.— Ki b kaj  was insignis Hook f. ernacular— Lep.—Serh; Nep.—	in the marshes of Peshawar and in the warm valleys of the Himalayas. It appears during the cold weather and remains until the break of the rains.  A small tree found in the interior valleys of the Sikkim Himalayas at altitudes of 3,000 to 6,000 ft., and in the Khasia Hills	Supplement 1935].	were Europ begga maint sores Leaves, juice

Name of plant	Distribution	Constituents	The part or parts of the plant which cause dermatitis
59. Rhus punjabensis J. L. Stew. ex. Brand. Vernacular— Pun.—Arkhar, Kakk ein, Titari	A small or medium-sized tree found in the North-Western Himalayas at altitudes of 2,500 to 8,000 ft. from the Indus eastwards and is common in the inner ranges in moist ravines, etc.		Leaves, bark, fruit. The juice is a vesicant.
60. Rhus succedanea Linn. English— Crab's claw, Japan wax tree, Red lac sumach Vernacular— Hi. and Be.—Kakra- singi; Bo.—Takada- singi; Pun.—Arkhol	A medium-sized tree found in the temperate Himalayas from Kashmir to Sikkim and Bhutan at altitudes of 3,000 to 8,000 ft. It also occurs in the Khasia mountains between 2,000 and 6,000 ft., and in Sind.	The leaves contain about 20 per cent of tannin. The milky juice yields a lac similar to Japan lac with laccol, a toxic phenol. Laccol is identical with urshiol [Wehmer, 1929-31 Supplement 1935].	Leaves, bark, fruit. The juice is a vesicant.
61. Rhus wallichii Hook. f. Vernacular— Hi.—Akoria; Nep.— Chosi; Pun.—Arkhar, Arkol	A small tree found in the temperate Himalayas from Garhwal to Nepal, occurring at altitudes of 6,000 to 7,000 ft. above sea level.		Leaves, bark, fruit. The juice possesses vesicant properties.
62. Rumex acetosa Linn. English— Dock sorrel, Sorrel	A perennial herb met with in the Western Himalayas from Kashmir to Kumaon, at altitudes of 8,000 to 12,000 ft.	The plant contains oxalates as well as free oxalic acid [Berthelot and Andre, 1886; Fleury, 1889]. It contains acid potassium oxalate and some tartaric acid [Watt and Brayer-Brandwijk, 1932]. Purdie [1927] found 1.36 per cent of potassium binoxalate in the juice. Maurin [1926] reports 1.05 per cent of oxymethylanthraquinone from the roots and traces of the same from the leaves.	Leaves produce dermatitis in susceptible persons.
63. Rumex acetosella Linn. English— Field sorrel, Sheep's sorrel, Sourack Vernacular— Be.—Chukapalam; Sans.—Chutrika	A perennial herb found in the Eastern Himalayas in Sikkim at altitudes of 7,000 to 8,000 ft.	The herb contains oxalates as well as free oxalic acid. Also contains potassium binoxalates [Orlandini, 1933].	Leaves
64. Ruta graveolens Linn., var. angustifolia Hook f. English— Common rue, Country- man's treacle, Garden rue Vernacular— Hi.—Pismarum, Sudab; Be.—Ermul, Ispund; Bo.—Satap	Occasionally cultivated in gardens	An essential oil	Leaves. If much handled they produce redness, swellings and even vesication of the part with which they come in contact.

Name of plant	Distribution	Constituents	The part or parts of the plant which caus dermatitis
65. Sapium insigne Trimen Vernacular— Hi.—Khinna; Bo.— Dudla; Pun.—Bileja, Dudla	A small or middle-sized tree found in the Sub-Himalayan tract and outer Himalayas from the Ravi eastwards to Bhutan (not in Sikkim) ascending to an altitude of 5,500 ft.; also in Assam, Chittagong and Orissa. In Western and Southern India it is common near the seacoast of Konkan and North Kanara, and is also found in the Deccan, hills of Kurnool, Cuddapah and Nellore, Kambakam Hill in Chingleput, Western Ghats and West coast, and is usually found in rocky places up to 6,000 ft. above sea level.		Milky juice is acrid and acts as a vesicant.
66. Schima wallichii Choisy. Vernacular— Hi.—Chilanni, Makusal	A large evergreen tree of the Eastern Himalayas, from Nepal and Sikkim to Bhutan, found at altitudes between 2,000 and 5,000 ft. It also occurs in Assam, the Khasia hills and Chittagong.	Leaves contain saponin [Wehmer, 1929-31, Supplement 1935].	The bark, in which the liber cells appear like glistening-white needles. irritates the skin in the same way as cowhage (Mucuna prurita).
137. Semecarpus anacardium  Linn. f. English— Common marking nut tree Vernacular— Hi. and Be.—Bhela; Bo.—Biba; Sans.— Bhall ttamu	A moderate-sized tree found in the Sub-Himalayan tract from the Beas eastwards, ascending in the outer hills up to 3,500 ft., Assam, Khasia Hills, Chittagong, Central India, Gujerat, Konkan, Southern Mahratta Country, Kanara and in deciduous forests of all districts in the Madras Presidency.	Earlier investigators suggested that the black corrosive juice of the pericarp contained a tarry oil consisting of 90 per cent of an oxyacid named anacardic acid and 10 per cent of a higher non-volatile alcohol called cardol. Naidu [1925] isolated catechol and a monohydroxy phenol which he called 'anacardol' besides two phenolic acids and a fixed oil from the kernel of the nut. Pillay and Siddiqui [1931] were unable to find either anacardic acid or cardol or catechol and anacardol as reported by previous investigators. They isolated the following constituents from the juice of the pericarp: (a) A monohydroxyphenol which forms 0.1 per cent of the extract, (b) Ano-dihydroxy compound forming about 46 per cent of the extract; and (c) A tarry, non-volatile corrosive residue forming 18 per cent of the nut.	Juice from pericarp and the tree-trunk. It is a powerful counter-irritant and vesicant and has been used by malingerers for producing ophthalmia and skin lesions, as also by others for imitating bruises in support of a false charge. Cases are known where the juice has been employed to cause injury to other persons.

	Name of plant	Distribution	Constituents	The part or parts of the plant which cause dermatitis
68.	Semecarpus travancoricus Bedd. Vernacular— Mal. — Avukarum; Tam.—Shenkəttal; Tel.—Natu səngota	A very large tree found in the evergreen forests of Tinnevelly and Travancore up to an altitude of 4,000 ft.		Juice; properties simila to that of S. anacardium
69.	Tragia bizolor Miq.	A slender climbing herb found in the Western Ghats, the Nilgiris and Pulney Hills at an altitude of 5,000 to 6,000 ft. in Shola forests.		Stinging hairs on plant
70.	Tragia involucrata Linn.  (with varieties in the Flora of Brit. Ind. which are now treated as distinct species, viz. T. hispida Willd., T. muelleriana Pax and Hoffm., T. cannabina Linn. f. and T. montana (Thw.) Muell-Arg.  Vernacular—  Hi.—Barkanta; Be.—  Bichuti; Bo.—Kanch-kuri; Sans.—Vrishchi			Stinging hairs on plant
71	kali  Urtica dioica Linn. English— Common nettle, Stinging nettle Vernacular— Hi. and Pun.—Bichu, Bichua	An erect herb found in the North-West Himalayas from Kashmir and the Salt Range to Simla at altitudes of 8,000 to 10,700 ft. and in Western Tibet at altitudes of 8,000 to 12,000 ft.	The plant contains lecithin and a glucoside [Wehmer, 1929-31, Supplement 1935]. According to Cleery [1927] the protoplasm of hairs has an alkaline reaction, and encloses an acid cell sap. The cell sap contains a small amount of formic acid as well as acetic, butyric and other volatile fatty acids. The specific poison of the cells, which is a non-volatile substance of an acid nature allied to the resin acids, is in solution in these acids.	Stinging hairs on plant
72	. Urtica hyperborea Jacq. Vernacular— Ladd.—Dzatsutt, Stokpotsodma, Zatud	A low, densely tufted under- shrub found in Western Tibet at altitudes of 12,000 to 17,500 ft. and in Eastern Tibet between altitudes of		Stinging hairs on plant
78	3. Urtica parviflora Roxb. Vernacular— Kum.—Berain, Bichhu, Shishona	ft. The flora of British India also records it from Ootacamund in the Nil-		Stinging hairs on plant
74	t. Urtica pilulifera Linn. English— Roman nettle	giris. A common European stinging weed occurring occasionally near Simla and elsewhere near habitations in the hills.	The seeds contain a fatty oil and a glucoside [Wehmer 1929.31, Supplement 1935].	Stinging hairs on plant

Name of plant	Distribution	Constituents	The part or parts of the plant which cause dermatitis
75. Wallichia disticha T. Anders. Vernacular—	A handsome palm of the outer hills of Sikkim	••••	Berries and perhaps also the leaves [Watt, 1889- 96]
Lep.—Katong  76. Xanthium strumarium Linn. English— Bur-weed, Cocklebur Vernacular— Hi.—C'hhota-gokru; Be.—Bon-okru; Bo.—Shankeshvara; Sans.—Arishta	A herb throughout the hotter parts of India, usually near habitations, ascending in the Western Himalayas to an altitude of 6,000 ft.	1.27 per cent of amorphous glucoside xanthostrumarin, etc. in seeds [Zander, 1881].	Leaves cause severe vesi- cular dermatitis in suscep- tible persons. Our plant collector is particularly sensitive to it. It is only, however, in the growing season that he suffers. In November when the plant is wither- ing he is not susceptible.

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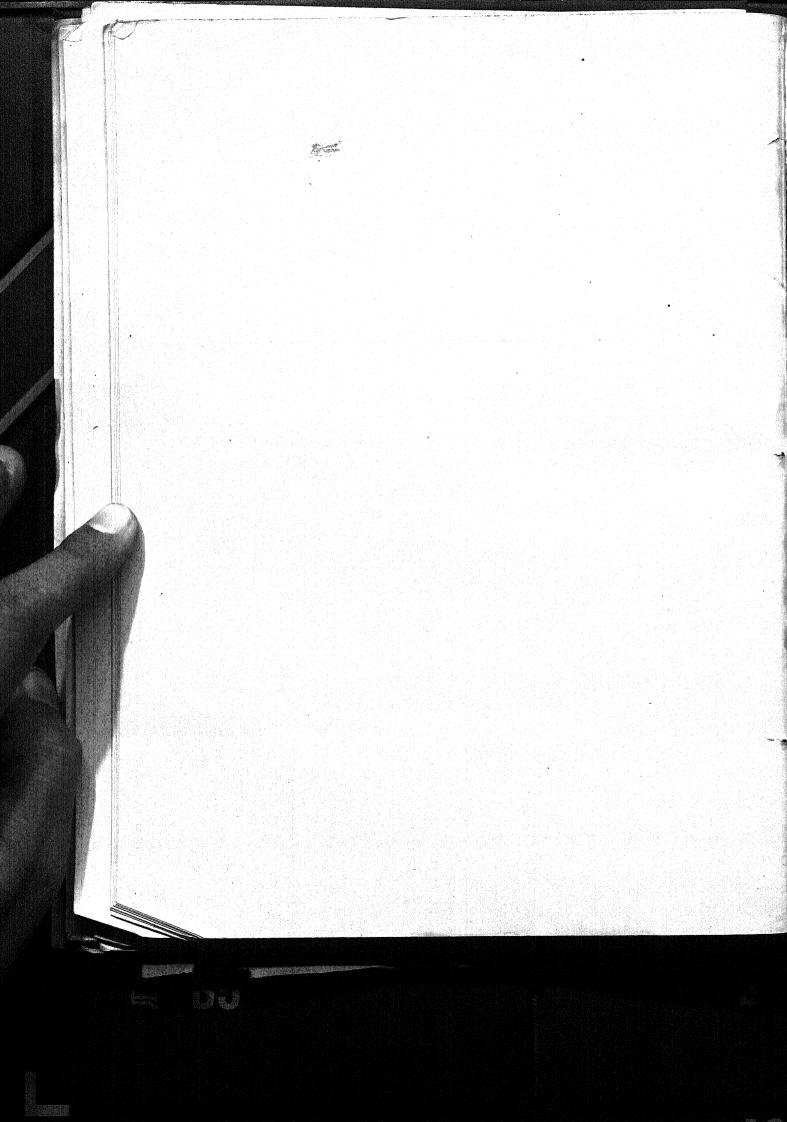
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LIBBBBB

## ORIGINAL ARTICLES

## NOMENCLATURE OF OLEIFEROUS BRASSICAS CULTIVATED IN THE PUNJAB\*

By ZAFAR ALAM, B.Sc. (AGRI.), M.Sc.(LONDON), Botanist for Oilseeds, Punjab Agricultural College and Research Institute, Lyallpur (Received for publication on 28 February 1945)

THE oleiferous brassicas cultivated in the Punjab, or in India, are divided into three groups, locally known as rai (mustard), sarson (colza) and toria (rape). In the Punjab, or for that matter in different provinces or tracts of India, the various forms of these three crops have definite local names which are readily understood in the provinces or tracts of their origin but are unintelligible to people of alien areas. Therefore when it is desired to make the identity of a particular plant known or understood over a wide area, it is a general rule to apply the scientific name to it. In some cases, however, the scientific nomenclature is highly confusing and it is not easy to decide as to what particular scientific name should be used for a certain plant. This confusion is partly due to the same plant having been given different scientific names by different workers ignorant of each other's labours, but as pointed out by Bailey [1922] the following causes, among others, appear to be mainly responsible for the chaotic nomenclature of brassicas:

(a) Inclusion of too many forms in one species,

thereby weakening the definitions.

(b) Attempts to identify oriental cultivated brassicas with species known in Europe.

(c) Mixing of seeds.

Bailey's paper deals mainly with the cultivated brassicas of China and Japan. Although he grew some of the Indian brassicas, he has not given any account of these as he agrees with the classification given by Prain [1898].

Prain, in his classic monograph, has described the confusion prevalent in his time with regard to the use of local and scientific names, and has straightened out the nomenclatorial tangle to a great extent. It has, however, been felt by later workers that the position in respect of at least two of the cultivated Indian brassicas, viz. sarson and toria, has not been sufficiently clarified.

The chief object of the present paper is to dispel the existing confusion and, in the light of the latest knowledge, to decide as to what scientific names should be applied to the brassicas cultivated

in the Punjab. And since the brassicas grown in this Province are also cultivated, to a greater or less extent, all over India, the decisions arrived at will naturally be applicable to the brassicas cultivated throughout India. It is not proposed to make, in this contribution, any definite statements with regard to the affinities or relationships of the plants under consideration.

# OLEIFEROUS BRASSICAS CULTIVATED IN THE PUNJAB

As already stated, the oleiferous brassicas cultivated in the Punjab are divided into three main groups—rai, sarson and toria. There are several characteristics peculiar to each group, but they can easily be distinguished from each other on the basis of leaf characters.

In rai, the leaves do not clasp the stem, while in sarson and toria, with the exception of the lowermost two or three, they are amplexicaul. In sarson the leaves, at least the lower ones, are always more or less hispid, and so also is the lower part of the stem, while in toria both stem and leaves are glabrous.

Three forms of rai, two of sarson and one of toria are recognized in the Punjab. Toria is sometimes sub-divided into two kinds (a) tall and late and (b) dwarf and early, but as will be shown later these differences are purely due to soil and climatic conditions and have no taxonomic status. Therefore from the standpoint of a systematic botanist there is only one form of toria.

#### . METHOD OF STUDY

Three forms of rai, two of sarson and one of toria were for some years grown at the Oilseed Breeding Farm, Lyallpur, and later for a season in the Experimental Area of Professor R. Ruggles Gates, F.R.S., in the Regent's Park, London. The plants belonging to different forms of these three crops were studied from germination till maturity. Specimens were taken at different stages of growth, and were later compared, for the purpose of identification, with the specimens stocked in the Royal Herbarium, Kew, where opportunity was also taken of discussing, with the taxonomists dealing with Indian plants, the best plan for

<sup>\*</sup>Part I (in a modified form) of a thesis approved for the Degree of Master of Science of the University of London.

satisfactorily solving the nomenclatorial tangle of Indian brassicas. It may be noted that in the present contribution only those characters have been taken into consideration which, through long observation and study under radically different environmental conditions, have been found to be constant and non-variable and thus to be of taxonomic value.

IDENTIFICATION OF OLEIFEROUS BRASSICAS CULTI-VATED IN THE PUNJAB

Rai (mustard)

The three forms of rai cultivated in the Punjab are locally known as (a) raya, (b) rai and (c) poorbi

(a) Raya. This is the plant which Roxburgh [1832] described as Sinapis ramosa, and which Hooker and Thompson [1861] described as Brassica juncea. It has been described by Prain on page 47 of his monograph as Brassica juncea subsp. juncea,

var. oleifera.

Prain thinks that this plant found its way into India from China through a north-eastern route and that its immigration to India has been independent of any Aryan incursion. Sinskaia [1928] has confirmed this view and has shown that the native country of B. juncea is Asia, the centre of diversity of forms being China. B. juncea is cultivated in Afghanistan as well and, after a comparative botanical study of various Afghanistan mustards, Vavilov and Bukinich [1929] have concluded that *B. juncea* has found its way into Afghanistan from India. All the above opinions point to the conclusion that B. juncea was originally introduced from China into north-eastern India whence it has gradually extended to Afghanistan, most probably via Punjab.

(b) Rai. This is Brassica tournefortii Gouan., which is the same thing as Brassica stocksii H.f. & Specimens of this plant were collected by Thompson from cultivated fields in 1843 near Ludhiana and in 1846 near Dharamkot (Ferozepur district). He also collected a solitary specimen near Jhang. Later in 1922, Mr J. R. Drummond sent another specimen from Sirsa (Hissar district). All these specimens are stocked in the Royal Herbarium, Kew. It may be noted that all the above-mentioned places of collection are situated

in the Punjab.

Prain has not given any detailed account of this plant in his monograph, as it was not found by him to occur in the then constituted province of Bengal-the area now covered by the provinces of Bengal, Bihar and Orissa. Sabnis and Phatak [1935] also have made no mention of this plant in their classification of cultivated Indian mustards presumably because this mustard is not grown in

the area covered by their studies. The information to hand shows that this plant has not been known to extend eastwards beyond Delhi and Ajmer. It has, however, been reported to be under cultivation in western Tibet and to extend westwards to Italy and Spain. In the Punjab itself this form is commonly grown on the borders of fields in the central districts, which may be regarded as centres of ancient agriculture. It, therefore, seems highly probable that it has been under cultivation in the Punjab for a very long time, perhaps much longer than the other two forms of rai. It appears to have been introduced into the Punjab through a north-western route.

Nothing definite is known about its origin but it is believed by Prain to have originated in the Oriental or Mediterranean areas. The recent intensive studies carried out by the Russian school of workers on some oleiferous plants have shown that cultivated mustards and colzas have originated from weeds, perhaps independently in different regions. It appears likely that this form, like Eruca sativa Lam., has two independent centres of

origin, Asiatic and Mediterranean.

(c) Poorbi rai. This plant is Brassica nigra Koch. It resembles in many respects the forms belonging to the geographical 'Eastern group' of Sinskaia, and in a greater degree the form described by her as B. nigra var. orientalis which is cultivated in Asia Minor.

With the present state of our knowledge it is not possible to venture any opinion about the introduction of this form into India. In the Punjab its cultivation is mainly confined to some parts of south-eastern districts, where it seems to have been introduced from districts situated farther

(d) Brassica alba. It is, at present, practically unknown in the Punjab. During the year 1926-27 an extensive and comprehensive collection of seedsamples of mustards was made from all over the Punjab, but none of these samples was found to be that of B. alba. Prior to 1927, however, this form used to be grown in the Botanical Experimental Area, Lyallpur, its seed having been originally obtained from outside the province. Hooker and others [1872] mention that Thompson came across cultivated fields of B. alba at Ferozepur in the Punjab. If this information is correct, the cultivation of B. alba must have ceased since, as now it is not grown at all in the Punjab.

Sarson (colza) and toria (rape)

Two forms of sarson, locally known as (a) pili (yellow-seeded) and (b) kali (brown-seeded) sarson, are cultivated in the Punjab. There exists a great deal of confusion about the naming of these forms and workers on these crops have, therefore, often

employed a diversity of botanical names while referring to these plants. So far as toria is concerned the present-day workers generally apply to this plant the name Brassica napus L., var. dichotoma Prain, but, as will be shown later, the use of this name is not valid. As toria resembles the brown-seeded form of sarson rather closely in morphological and some physiological characters, it is proposed to discuss it along with the two forms of sarson.

The yellow-seeded form of sarson cultivated in the Punjab has two-chambered, erect pods. In Floras of India, however, forms with three-valved, nodding fruits, and with four-valved, erect fruits have also been described. This multi-valved character of the fruit has been given specific rank by some workers. Prain in his monograph has discussed in detail the taxonomic value of this character, and the author has also seen specimens in the Royal Herbarium, Kew, and in the extraprovincial collection of yellow-seeded sarson at Lyallpur, where two- and four-valved pods occur on the same plant. He, therefore, agrees with Prain that, other things being equal, these differences are not more than racial. Prain's collection also included a two-valved, nodding-fruited form, which had not been mentioned by any previous worker.

It has already been stated that the taxonomists disagree widely in the systematic treatment of these brassicas. The following is a brief account of the names applied by different taxonomists to yellow-seeded and brown-seeded forms of sarson, and to toria.

Roxburgh treated the two-valved, erect-fruited form of yellow-seeded sarson as one species (Sinapis glauca) and the nodding-fruited, three-valved form as another (S. trilocularis). He named toria as S. dichotoma.

Hooker and Thompson lumped the two-valved, erect-fruited form of yellow-seeded sarson (S. glauca of Roxburgh) and toria (Roxburgh's S. dichotoma) into one group and referred to it as Brassica campestris. Later, Hooker and others termed it as Brassica campestris subsp. napus. They considered the 3-4-valved, nodding-fruited form as one species (Brassica trilocularis) and the 4-valved, erect-fruited form as another (B. quadrivalvis).

Duthie and Fuller [1882] regarded the two-valved, erect-fruited form of yellow-seeded sarson as B. campestris subsp. napus, var. glauca; the 3-4-valved, nodding-fruited form as var. trilocularis, and the four-valved, erect-fruited form as var. quadrivalvis. They also regarded brown-seeded sarson and toria as varieties of B. campestris subsp. napus and christened them var. dichotoma and var. toria, respectively. Their var. dichotoma,

so these workers say, is the S. dichotoma of Roxburgh. Actually, however, Roxburgh's S. dichotoma is not precisely the equivalent of Duthie and Fuller's var. dichotoma, as Roxburgh's description applies to toria which has glabrous leaves, and Duthie and Fuller's to brown-seeded sarson which has hispid leaves. In fact, Roxburgh does not seem to have described the brown-seeded form of sarson at all. Duthie and Fuller in this case seem to have placed too much reliance on the judgment of Royle [1839], who quoted the name of S. dichotoma Roxb. for kali (brown-seeded) sarson. Royle's quotation of synonyms and his judgment, however, are faulty as he names toria as S. glauca Roxb., which latter appellation is applicable only to a form of yellow-seeded sarson. Furthermore, Duthie and Fuller's description of var. dichotoma does not refer to the taller and later kind of toria as Prain supposes. It without doubt refers to brown-seeded form of sarson which has hispid leaves. No doubt two kinds of toria are sometimes recognized, but none of them ever has hispid leaves. In the Punjab a kind of toria known as sathri is cultivated in some parts of the Multan district. This kind differs from ordinary toria in being shorter in stature and earlier in maturity and also in having slightly smaller leaves. The author has had the opportunity of growing and studying this form at Lyallpur, where taller and later kind of toria is exclusively grown, and also of growing and studying the latter kind at Multan. It was found that at both these places the newly introduced sorts (grown in sequestered places to prevent cross-pollination with other sorts) soon lost their identity and attained a close conformity with the native sort, showing thereby that the differences in size and maturity were due to climatic and/or soil conditions. Duthic and Fuller's description of var. toria, of course, fits in better with the dwarf and earlier kind, but, as is clear from the case cited above, dimensional proportions alone are of no taxonomic value in this case.

Watt [1889] mainly adopted the nomenclature proposed by Duthie and Fuller, but suggested that brown-seeded sarson, on account of having hispid leaves, should be placed in subsp. campestris, and that toria, on account of having glabrous leaves, should be viewed as belonging to subsp. napus. He, however, erroneously regarded Duthie and Fuller's vars. trilocularis and quadrivalvis as abnormal forms of Roxburgh's S. dichotoma.

Prain combined the forms of yellow-seeded sarson having (a) 2-valved, erect fruits, (b) 2-valved, nodding fruits, (c) 3-4-valved, nodding fruits, and (d) 4-valved, erect fruits, with the brown-seeded sarson into one comprehensive group which he called Brassica campestris var. sarson.

In the systematic synopsis in his monograph he treated toria as Brassica campestris subsp. napus var. oleifera, but adopted the term dichotoma on account of its priority. Further, because of marked differences in the time of growth, mode of cultivation, etc., of sarson and toria he considered it expedient to regard toria (subsp. napus) as a distinct species. The upshot of all these considerations was that he finally named toria as Brassica

napus var. dichotoma.

Later, Duthie [1903] abandoned the nomenclature he had previously proposed in collaboration with

Fuller and adopted the names suggested by Prain. But he applied the name B. campestris L., var. sarson Prain, to the yellow-seeded sarson only, and B. napus L., var. dichotoma Prain, to both brown-seeded sarson and toria. This use of a single appellation for both brown-seeded sarson and toria by Duthie was probably the outcome of the erroneous statement made by Prain (already mentioned) that var. dichotoma of Duthie and Fuller (which actually referred to brown-seeded sarson) was the taller and later kind of toria.

Once again, Duthie seems to have been misled by the wrong quotation of synonymy.

That Prain's B. campestris var. sarson refers to both yellow- and brown-seeded forms of sarson is clear from the fact that Prain described the seed colour as 'dingy white, yellow or brown'. further described sarson as a hairy-leaved, and toria as a glabrous-leaved, sort. Furthermore, he stated that sarson is cultivated mixed with other crops, and that toria is always grown alone. All these statements point definitely to the conclusion that he included both the yellow- and brownseeded forms in the epithet B. campestris var. sarson. This has also been the opinion of most workers in India. For example, the specimens of both yellow-seeded and brown-seeded sarson sent by the Imperial Economic Botanist, Pusa, to the Royal Herbarium, Kew, were labelled B. campestris var. sarson, Prain. Mohammad, Singh and Alam [1931] and several other workers have also applied the name B. campestris var. sarson Prain, to both these forms of sarson.

For the sake of quick comprehension different systematic names that have been applied to yellow-seeded sarson, brown-seeded sarson and toria by various taxonomists are given in

Table I.

### DIFFERENCES BETWEEN YELLOW-SEEDED SARSON, BROWN-SEEDED SARSON AND TORIA

For facility of presentation differences between yellow-seeded sarson and brown-seeded sarson have been first enumerated and set out in Table II, and then the differences between brown-seeded sarson

and toria have been recounted and given in Table III.

TABLE I

Some of the systematic names that have been applied to yellow-seeded sarson, brown-seeded sarson, and toria

Authority	Yellow-seeded sarson	Brown-seeded sarson	Toria
Roxburgh	Sinapis glauca S. trilocularis	S. dichotoma?	S. dichotoma
Hooker and Thompson	Brassica campestris B. trilocularis	B. campestris	B. campestris
	B. quadrivalvis		
Hooker and others	B. campestris, sub- sp. napus	B. campestris, subsp. napus	B. campestris, subsp. napus
	B. trilocularis° B. quadrivalvis		
Duthie and Fuller	B. campestris, subsp. napus, var. glauca	subsp. napus,	B. campestris, subsp. napus, var. toria
	B. campestris, sub- sp. napus, var. tri- locularis		
	B. campestris, sub- sp. napus, var. qua- drivalvis		
Watt	B. campestris, subsp. napus, var. glauca	subsp. cam-	B. campestris, subsp. napus, var. toria
	Regards vars. tri- locularis and qua- drivalvis as abnor- mal forms of Rox- burgh's S. dicho- toma		
Prain	B. campestris, var.	B. campestris	B. napus, var dichotoma
Duthie	B. campestris, var.	B. napus, var dichotoma	B. napus, var dichotoma

#### DISCUSSION AND CONCLUSIONS

The extrorse or introrse nature of anthers seems to have escaped the notice of all taxonomists and early workers dealing with sarson and toria plants. It was in 1931, that Mohammad, Singh and Alam [1931] first noted this characteristic difference.

Prain, while lumping both yellow- and brownseeded forms of sarson in one comprehensive species, did not attach any value to the colour of seeds, most probably due to the fact that his work related to Bengal where yellow-seeded form is almost exclusively grown and he, therefore, did not

#### TABLE II

Differences between yellow-seeded and brown-seeded forms of sarson

Vellow-seeded sarson

Brown-seeded sarson

#### 1. Lowermost 1 - 2 leaves

Lamina prominent up to the Lamina practically absent very base of the leaf. very base of the leaf.

#### 2. Colour and texture of leaves

Dark glaucous, fleshy.

Pale glaucous, thin.

### 3. Branching

Branches erect, ascending, Straggling plants absent. The angle at which the primary branches arise varies from 10° to 20°.

Rather erect to spreading. Straggling plants occasionally present. The angle at which primary branches arise varies from 23° to 43°.

#### 4. Corolla

Diameter from  $14 \times 15$  mm. to  $17 \times 16$  mm. Average length of claw plus blade 10.2 mm., length of claw 3.2 mm., and width of blade 5.1 mm. Potals narrow, with spaces between the adjacent ones.

Diameter from  $15 \times 15$  mm. to 20×19 mm. Average length of claw plus blade 11.4 mm., length of claw 3.6 mm., and width of blade 7.1 mm. Petals broad, 7·1 mm. Petals generally overlapping.

#### 5. Anthers

In the bud as well as in the open flower all the six anthers introrse.

In the bud all six anthers introrse, but in an open flower the anthers of four median stamens extrorse, of two lateral stamens introrse.

#### 6. Pods

Thick and broad, never torulose.

Thin and narrow, sometimes torulose.

#### 7. Seeds

mucilaginous.

Dingy white, or yellow; non- | Dark brown, brown, or reddish brown; mucilaginous.

#### 8. Fertility

Self-fertile.

Highly self-sterile.

#### 9. Maturity

flowering and maturity.

At least a week later in | At least a week earlier in flowering and maturity.

#### 10. Localities of cultivation

Grown only in parts of Rawalpindi and Kangra districts of the Punjab.

Grown almost all over the Province.

#### TABLE III

Differences between brown-seeded sarson and toria

Brown-seeded sarson

Toria

#### 1. Leaves and stem

At least the lower leaves more or less hairy, and so also the lower part of the stem; leaves thin.

Leaves and stem glabrous: leaves somewhat fleshy.

#### 2. Seeds

generally Mucilaginous; there is a preponderance of darker coloured seeds in a sample.

Non-mucilaginous; generally lighter coloured seeds predominate.

#### 3. Maturity

When sown at the same time as toria, at least a fortnight later in flowering and maturity. In the Punjab it is a rabi (spring) crop, being sown in October-November and harvested in March-April.

When sown at the same time as brown-seeded sarson, at least a fortnight earlier in flowering and maturity. In the Punjab it is a zaid kharif (autumn) crop, being sown in September and harvested in December-January.

#### 4. Cultivation and uses

Grown almost all over the Province mixed with other crops, mostly on barani (rainfed) areas. In addition to the extraction of oil from its seeds, used as fodder and vegetable. Oil is preferred for culinary purposes.

Always grown alone, almost entirely on irrigated areas. Exclusively grown for the extraction of oil from its seeds. Plants are not relished as vegetable, and oil is used for culinary purposes only if sarson oil is not available.

have an opportunity of studying thoroughly the brown-seeded form of sarson. No doubt he found some brown seeds in the samples of yellow-seeded sarson collected by him, but he erroneously concluded that since both sorts of seeds occurred in the same sample, the seed colour was of no taxonomic value. As pointed out by Bailey [1922], there is a need for great caution in formulating an opinion from the examination of seed-packets The seeds obtained from market or from some other dubious source, in most cases, contain a mixture of more than one seed forms, and this mixing of seeds has been greatly responsible for the confusion prevalent in the nomenclature of cultivated brassicas. Prain's conclusion that seed colour is of no great import in the classification of brassicas is probably the outcome of this mixing of seeds. Duthie and Fuller also seem to have been misled by the same cause because while

describing the yellow-seeded sarson they mention the colour of seeds to be 'yellow, dark brown, or reddish brown'. To the same cause may be traced the erroneous conclusion that the forms of cultivated brassicas at times pass into one another. From his long experience of breeding work on yellow-seeded and brown-seeded forms of sarson, the author is convinced that if care is taken to avoid accidental mixing of seeds, the progeny of yellow or brown seeds is always 'true to type'. Studies carried out by some workers [Mohammad, Sikka and Aziz, 1942] have also shown that yellow and brown colours of seeds in the case of sarson are heritable characters. There is no doubt that on account of the prevalence of unfavourable weather conditions during maturity, or during postharvest period when the crop is still lying in the field awaiting threshing, or through long storage the colour of seeds undergoes some change, but this change is not so radical as to lead to a confusion of identities and to give rise to the possibility of yellow seeds being mistaken for brown seeds. Therefore, there is ample evidence to believe that colour of seeds (yellow or brown) is, in the case of sarson, a good taxonomic character.

The confusion prevalent as regards the nomenclature of yellow-seeded sarson, brown-seeded sarson and toria has already been described in some detail in the preceding pages. From the differences enumerated in Tables II and III, it will be seen that the yellow-seeded sarson differs markedly from the brown-seeded sarson, or for the matter of that from toria, in morphological, physiological and ecological characteristics. Furthermore, the idea that intermediate forms sometimes exist is not supported by any authentic and unassailable evidence. Therefore, the yellow-seeded sarson deserves a specific rank and should be singled out as a separate species instead of being lumped with brown-seeded sarson. Sinskaia has also expressed

the same opinion.

The differences between the brown-seeded sarson and toria, on the other hand, are not so great as to entitle them to be classed as different species, and both of these should, therefore, be treated as belonging to a single species. The next point to decide is as to whether these should be placed in species B. napus or B. campestris. Linne [1763] distinguished B. napus and B. campestris on the character of the root—'fusiformi' for napus, and 'tenui' for campestris. Later workers, however, have realized that this delimitation of the two species is not enough and has been a cause of great confusion. Sinskaia, after a detailed study, has given a list of characters on which the delimitation of the species B. napus and B. campestris should be based. A few of these characters, germane to the points at issue, are given in Table IV.

TABLE IV

Characters of the species B. napus and B. campestris

B. napus	B. campestris
Self-fertile	Fertility   Highly self-sterile
	Chromosome number
2n=36	2n=20 Pubscence

Strongly pubescent forms | Strongly pubescent forms absent | present

Crossability with B. glauca Wittm.\* (yellow-seeded sarson)

Does not cross | Crosses readily

Geographical conditions

Concentrated almost exclusively in Europe Widely spread in Asia

Nagaharu and Nagamatsu [1932] tested the fertility of varieties of B. napus and B. campestris, and confirmed that the former is self-fertile, while the latter is mainly self-sterile.

Both brown-seeded sarson and toria are highly self-sterile, have chromosome number 2n=20, and cross readily with yellow-seeded sarson. They both should, therefore, be treated as belonging to the species Brassica campestris.

The yellow-seeded sarson is self-fertile and has chromosome number 2n=20, so that it partakes of some characteristics of both B. napus and B. campestris and, therefore, is in a class by itself. This is an additional reason for separating it as a distinct species.

Nomenclature. Having decided to regard the yellow-seeded sarson as a separate species and to treat brown-seeded sarson and toria as belonging to the species B. campestris, the next question is to decide as to what name should be applied to each of them.

Sinskaia has used the name Brassica glauca Wittm. for yellow-seeded surson on the authority of Dr Wittmack, but she has made no reference to any publication wherein Dr Wittmack first applied this name to this form of sarson. Prain, while quoting, in his monograph, the synonym B. glauca Wittm. cited the Kew Report by Hooker [1877] as the source of his information. In this Report, however, the only statement that has been made by Hooker in this connexion is to the effect that Dr Wittmack has identified the seed of Guzerat Rape as the seed of S. glauca Roxh., and it is nowhere stated by Hooker that Dr Wittmack has applied the name B. glauca to

\*As will be shown later the use of name B. glauca Wittm. for yellow-seeded surson is not correct.

the yellow-seeded sarson. In the Index Kewensis also there is no mention of the name B. glauca as having been used by Dr Wittmack in connexion with some plant. The name B. glauca, as stated by Durand and Jackson [1906] in Index Kewensis, was first applied by Kuntze [1891] to a plant found in the Canary Islands. Evidently, therefore, Prain was mistaken in assuming that the name B. glauca was applied by Dr Wittmack to the yellow-seeded sarson, as the evidence available does not justify such an assumption. Sinskaia probably used this name on the authority of Prain. Since the name B. glauca has already been used by Kuntze for another plant, the application of this name to the yellow-seeded sarson is not at all valid. In order to decide as to what name should be applied to this form of sarson recourse must be had to the list of names given in Table I, and out of the several names listed there for this sarson, one having priority of application should be selected. It will be seen that Roxburgh was the first taxonomist to apply the name S. trilocularis to the form of yellow-seeded sarson having 3-celled pods. Later, Hooker and Thompson called the same form Brassica trilocularis. No doubt in both these cases this form was treated as a distinct species, and the later work has shown it to be only a race. But the fact remains that the appellation trilocularis has been used specifically to designate yellow-seeded sarson in some way, and therefore it must be adopted on account of its priority. Since the old generic distinction into Sinapis and Brassica has been given up on account of its artificiality and the practice now is to lump the two old genera into one, under the name Brassica, hence Brassica trilocularis H.f. & T. is the name that should be applied to the yellow-seeded sarson. It should, however, be considered to include all kinds whether they have 2-, 3-, or 4-valved and erect or nodding pods. It is realized that the use of the word trilocularis is not quite appropriate and is misleading to a certain extent but it has to be retained on account of its priority. In fact International Rules of Botanical Nomenclature [Briquet, 1935] outlaw the use of any other name but this for the yellow-seeded sarson. As pointed out by Higgins [1937] the only way to clear up the tangle of nomenclature is to follow these rules. faithfully, and if some of the names which have been in use for a long time and have thus become familiar are found to be wrong according to these rules the only course to help towards a happier future and to bring order out of chaos is to accept

the change with a good grace.

With regard to the brown-seeded sarson and toria, it has already been shown that they should be treated as belonging to the species Brassica campestris. With the present state of our know-

ledge it is not yet possible to appraise fully the taxonomic value of the differences in the cultivation of crops. Sinskaia has suggested the term cultivated ecotypes' for the forms differing in their mode of cultivation. An ecotype, from her point of view, is equivalent to a 'variety'. The present-day tendency [Stout, 1940], however, is to assign to a group a rank commensurate with the differences it exhibits from other allied groups. Applying this basis and taking into consideration all the differences mentioned in Table III, it appears best to treat the brown-seeded sarson and toria as belonging to different subspecies of a single species. Watt suggested that the brown-seeded sarson should be regarded as subspecies campestris, and toria as subspecies napus of B. campestris. This suggestion of his fully meets the requirements of the point under consideration and should, therefore, be accepted. Hence the brownseeded sarson should be christened as Brassica campestris L., subsp. campestris, var. dichotoma Watt., and toria as Brassica campestris L., subsp. napus, var. toria D. & F. Since the latter name was first proposed by Duthie and Fuller, it has been retained on their authority. Diagnostic characters, brought up-to-date, of yellow-seeded sarson, brown-seeded sarson and toria are given in Appendix I.

In conclusion it may be pointed out that in view of the recent work on the delimitation of B. campestris and B. napus and in view of the edicts of the International Rules of Botanical Nomenclature, Prain's nomenclature B. campestris var. sarson for both the yellow-seeded and the brown-seeded forms of sarson, and B. napus var. dichotoma for

toria should be abandoned. As regards the introduction of these forms into the Punjab, it may be noted that recent and exhaustive studies carried out by the Russian workers have shown that eastern Afghanistan together with the adjoining north-western India is one of the independent centres of origin of B. campestris. From the fact that both the brownseeded sarson and toria resemble rather closely one or other of the groups of Afghanistan B. campestris isolated by Sinskaia, and from the availability of Persian synonyms for these crops, it is pretty certain that both brown-seeded sarson and toria have been introduced from the north-west into the Punjab, wherefrom they have extended eastwards into India.

The yellow-seeded sarson, on the other hand, is more commonly grown in the eastern provinces of India, where it exhibits a diversity of form. It is highly probable that further work might reveal its centre of origin in north-eastern India or in China. In the Punjab, however, it appears to have been introduced from the east.

#### SUMMARY

1. The three oleiferous brassicas cultivated in the Punjab are locally known as rai, sarson and

2. Three forms of rai are cultivated in the Punjab and bear the local names (a) raya, (b) rai and (c) poorbi rai. These have been identified as (a) Brassica juncea H. f. & T., (b) Brassica tournefortii Gouan., and (c) Brassica nigra Koch., respectively.

3. The appalling confusion prevalent about the nomenclature of two forms of sarson, viz. (a) yellowseeded and (b) brown-seeded, and of toria has been described, and the validity of scientific names proposed for these plants by various taxonomists

has been discussed.

Differences between these plants have been enumerated and it has been shown that the yellowseeded sarson should be classed as a distinct species, and that it is not valid to apply to it any other name than Brassica trilocularis H. f. & T. The brown-seeded sarson and toria, on the other hand, should be regarded as subspecies of B. campestris and the names Brassica campestris L., subsp. campestris, var. dichotoma Watt., and Brassica campestris L., subsp. napus, var. toria, D. & F., should be applied, respectively, to these plants.

It has been suggested that Prain's nomenclature for the two forms of sarson and for toria, which has gained general acceptance, should be abandoned as it (besides being out of tune with the latest taxonomic knowledge) runs counter to the procedure suggested in the International Rules of

Botanical Nomenclature.

4. Brassica juncea H. f. & T., Brassica nigra Koch., and Brassica trilocularis H. f. & T. appear to have been introduced into the Punjab through an eastern route; while Brassica tournefortii Gouan., Brassica campestris L., var. dichotoma Watt., and Brassica campestris L., var toria D. & F. are immigrants from the north-west.

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#### APPENDIX I

DIAGNOSTIC CHARACTERS OF THE YELLOW-SEEDED SARSON, THE BROWN SEEDED SARSON AND TORIA

(a) Brassica trilocularis H. f. & T. vern. pili (yellowseeded) sarson

An annual, 3-5 ft. high, plant with ascending branches. Root slender, tapering. Leaves, lamina extending to the base in all; the lower lyrate-pinnatipartite, upper smaller, lyrate-sinuate to lanceolate, entire; all except the lower 3-4 auricled, stem-clasping; dark green, glaucous, rather fleshy, more or less hairy—at least the lower ones. Flowers in oblong corymbs elongating into racemes. Sepals dark green, glaucous, turning yellow before falling. Petals yellow, narrow, about 0.5 cm. broad, well separated from one another. Anthers all introrse in bud and flower. Pod laterally compressed, much broader than thick, about 3-4 valved, erect or pendent; valves thickly leathery; beak conical, stout. Seeds sub-globose, dingy white or smooth. non-mucilaginous. Self-fertile. yellow, nearly smooth, non-mucilaginous. Late in maturity.

(b) Brassica campestris L., sub-sp. campestris, var. dichotoma Watt. vern. kali (brown-seeded) sarson

An annual, 3-5 ft. high, plant with ascending, medium, or spreading branches. Root slender, tapering. Leaves, lamina in the lowermost 1-2 practically absent in the basal half; the lower lyrate-pinnatipartite, decreasing upwards, upper smaller, triangular-lanceolate, entire; all except the lower 3-4 auricled, stem-clasping; green, glaucous, thin, more or less hairy—at least the lower ones, and also the lower part of the stem. *Flowers* in oblong corymbs elongating into racemes. Sepals green, glaucous, turning yellow before falling. Petals bright yellow, rarely pale yellow to whitish, broad, about 0.7 cm. wide, generally overlapping. Anthers in the bud all introrse, in the open flower those of the four median stamens extrorse, of the two lateral ones introrse. Pods rather narrow, about 0.5 cm. broad,

sometimes torulose; 2-valved, erect; valves thin; beak narrowly conical. Seeds sub-globose, dark brown, brown, or reddish brown, finely rugose, mucilaginous. Highly self-sterile. Late in maturity.

(c) Brassica campestris L., sub-sp. napus, var. toria D. & F. vern. toria.

An annual, 2-5 ft. high, plant with ascending, medium, or spreading branches. Root slender, tapering. Leaves, lamina in the lowermost 1-2 practically absent in the basal half; the lower lyrate-pinnatipartite, decreasing upwards, upper smaller, triangular-lanceolate, entire; all except the lower 3-4 auricled, stem-clasping; green,

glaucous, somewhat fleshy, quite glabrous, so also the stem. *Plowers* in oblong corymbs elongating into racemes. Sepals green, glaucous, turning yellow before falling. Petals bright yellow, rarely pale yellow to whitish, broad, about 0.7 cm. wide, generally overlapping. Anthers in the bud all introrse, in the open flower those of the four median stamens extrorse, of the two lateral ones introrse. Pods rather narrow, about 0.5 cm. broad, sometimes torulose; 2-valved, erect; valves thin; beak narrowly conical. Seeds sub-globose, dark brown, brown, or reddish brown, finely rugose, non-mucilaginous. Highly self-sterile. Early in maturity.

### A NOTE ON THE ESSENTIAL OIL OF MUSTARD IN BRASSICA SPECIES AND ERUCA SATIVA

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THE seeds of Brassicae are known to contain a glucoside called sinigrin or potassium myronate. On hydrolysis, under the influence of an enzyme, myrosin, which is also localized in the different cells of the seeds, this glucoside yields dextrose, essential oil of mustard (chemically termed allyliso-thio-cyanate), and potassium hydrogen sulphate according to the following reaction:

 $C_{10}H_{16}O_{9}NS_{2}K + H_{2}O = C_{6}H_{12}O_{6} + C_{3}H_{5}NCS + KHSO_{4}$ Water Dextrose Allyl-iso- Potassium Sinigrin thio-cyanate hydrogen

The essential oil of mustard is colourless, has an intensely pungent odour and an acrid taste. It is a powerful irritant, and causes very rapid vesication when applied to the skin.

The distribution of sinigrin in the different species of *Brassica* has a great economic importance. The disinclination shown by cattle to feed on green fodder of certain oleiferous Brassicae or on certain mustard seed cakes is mainly due to the irritating effects of this essential oil. The determination of mustard oil in seed cakes is, therefore, of considerable practical importance.

During the last few years the Oilseeds Section at Lyallpur, has been engaged on the evolution of new varieties of oilseeds and the funds provided by the Imperial Council of Agricultural Research have given impetus to this work. As a result of preliminary studies carried out an attempt has been made to classify the different species concerned according to their essential oil content, and the present note besides suggesting a new line of work

is intended to describe the results obtained during the two years 1942-43 and 1943-44.

A review of literature shows that very little work has so far been done on this subject. Athawale, Hare Duke and Mathur [1938] published some results on the industrial aspects of the production of fixed oils with a desirable pungent smell in compliance with the demand of the Indian markets, but these have a remote bearing on the present problem.

METHOD OF ESTIMATION AND SAMPLING OF PLANT MATERIAL

The volatile oil of mustard is prepared by distillation of the ground material macerated with tepid water for some hours so as to allow the myrosin to hydrolyse the glucoside present [Allen 1930]. In the present studies the method employed for the estimation was the same as that described by Athawale et al [1938] with slight modifications to suit the conditions of the experiment.

Five gm. in case of the seed and 25 gm. in case of stem, branches and leaves were thoroughly crushed and mixed with about 1 gm. of Kieselguhr to destroy the cellular structure. The mixture was transferred into a conical flask with 100 c.c. of water and kept at a temperature of 30°-40°C. for about four hours during which period 20 c.c. of alcohol were added to prevent frothing. The mixture was then distilled as rapidly as possible into 10 c.c. of 10 per cent ammonia till 2/3rd of the water content of the mixture was distilled over. With ammonia the essential oil forms a solid non-volatile compound. Twenty c.c. of  $\frac{N}{10}$ silver nitrate were then added and the mixture

Percentage of allyl-iso-thio-cyanate (essential oil of mustard) on dry basis in the developing ovules of Brassica species and taramira crops (average of two years 1942-43 and 1943-44) TABLE I

٠	To	ria	Brown	Brown sarson	Raya L-16	L-16	Raya L-18	L-18	Taramira	mira
Number of days after flowering	Percentage of moisture	Percentage of essential oil of mustard	Percentage of . moisture	Percentage of essential oil of mustard	Percentage of moisture	Percentage of essential oil of mustard	Percentage of moisture	Percentage of essential oil of mustard	Percentage of moisture	Percentage of sesential oil of mustard
	n	86.1	. 18	60.1	77.8	1.91	76.6	2.17	76.8	2.21
10 days	9:50	07 °C	78.5	2.5	82.3	1.84	6.92	1.29	81.1	1.26
" 20°	. o. ar	0.83	77.3	0.68	77.3	0.97	72.4	0.94	81.2	T.04
., 26,	2.62	9.60	68.0	89.0	6.69	96.0	2.09	86.0	74.2	96.0
40 ,,	09.T	0.76	49.0	89.0	62.5	1.10	57.5	1.05	64.3	0.93
, De	3 5	9.60	18.5	0.42	54.0	98.0	47.7	0.85	61.3	0.64
	e 10 F1 - 3	0.46	8.00	0.46	50.3	89.0	21.4	0.72	57.3	0.16

Percentage of allyl-iso-thio-cyanate (essential oil of mustard) on dry basis in various parts of Brassica species and taramira crops at various stages of their growth (average of 2 years 1942-43 and 1943-44)

		$T_o$	Toria	Brown sarson	sarson	Raya L-16	L-16	Rayo	Raya L-18	Tara	Taramira
Stage of plant at sampling	Name of the sample	Percentage of moisture	Percentage of essential oil of mustard								
Tender stage Leaves	Leaves	89.3	0.59	6.68	0.52	0.68	0.71	8.68	0.62	1.06	0.72
Young stage Stem	Stem Branches	93.5	0.62	93·2 92·4	0.62 0.49	93.0 92.3	0.86	94·8 93·2	0.00	92.9	0.59
	Leaves	8.68	0.35	2.06	0.34	87.4	0.45	91.1	0.47	e.06	0.33
T	Stom	88.1	0.34	8.68	0.34	91.2	09.0	90.3	0.47	91.6	0.39
blooming	Renches	88	0.52	89·I	0.25	92.2	0.20	91.4	0.42	2.16	0.41
stage	Leaves	0.88	0.34	89.2	0.30	87.4	0.54	89.4	0.42	7.88.	62.0
		00.00	0.19	85.6	0.13	81.6	0-21	80.1	0.25	81.7	0.22
Mid bloom-		70	76.0	6.68	0.50	9.08	0.32	8.62	0.27	82.3	0.54
agers gill	Leaves	88.5	0.17	85.7	0.12	87.3	0.22	81.6	0.25	87.0	0.23
تهريع دو	, de	70	0.13	 83.57	0.10	73.4	0.13	0.08	0.18	83.5	0.15
blooming	Branches	76	0.11	81.9	0.11	69.7	0.13	0.62	0.18	S 100	6.13
stage		86.5	0.14	83.7	0.11	84.7	0.21	6.78	6T.O	0.48	er o
Matroine	Ctom .		90.0	75.8	0.07	74.4	80.0	77.2	90.0	777.4	80.0
Marching	Decim	3 6	90.0	77.5	0.07	9.69	80.0	71.1	80.0	1.1.1	90.0

heated on a water bath for an hour. The silver sulphide produced was separated by filtration and the filtrate was rendered slightly acidic. The excess of silver was then titrated with 10 ammonium thiocyanate solution. Each c.c. of N silver nitrate consumed corresponds to 0 004956 gm. of

allyl-iso-thio-cyanate. The material for this investigation was obtained from toria (Brassica napus L. Var. dichotoma Prain), raya L-16 and L-18 (Brassica juncea), rai (Brassica nigra) and taramira (Eruca sativa) crops. Freshly opened flowers in a sufficiently large number were tagged in the case of each of the above crops and samples of developing ovules of known ages were obtained regularly at intervals of 10 days each. Besides the developing ovules, estimations of the essential oil were also made in leaves, stems and branches at various stages of their growth. For this purpose five plants were removed from each plot between 9 and 10 a.m. on each day of sampling and were divided into stem, branches and leaves. The results of these estimations (average of two years' data) are given in Tables I and II from which the following general conclusions are drawn:

(1) The essential oil content of the developing ovules (Table I), irrespective of the species, is the highest at the beginning of seed development and it goes on decreasing as the seeds advance in age. In the young ovules it has been found to be about three times the amount present near

the time of maturity. (2) Among the various species studied it was found (Table I) that raya L-16 and L-18 and taramira crops contained in the developing ovules a higher amount of essential oil at all the stages of their development than toria and brown sarson. This shows that the former crops contain a higher amount of sinigrin in the seeds at their various stages of development and this is responsible for their comparatively greater pungency.

(3) The mature seeds of Brassica species may be arranged in the following order in accordance with the degree of their pungency as determined

by the essential oil percentage: Rai (1.01), raya L-18 (0.80), raya L-16 (0.70),

toria (0.62), Brown sarson (0.60). It may, however, be noted that taramira contains the same amount of essential oil (0.71) as raya, possessing characteristic smell which can be easily distinguished from that of essential oil present in seeds of various Brassicae referred to above.

(4) Tender leaves contain a higher amount of essential oil than mature ones, it being about four times the amount present in the plants at the end of the blooming stage. Among the various species studied it was found that the essential oil in raya varieties is slightly higher than in toria and brown sarson. This difference is more pronounced in the later stages of growth than in the earlier. For example, in the young stages of growth the percentage excess in the essential oil in raya and taramira crops is about 23 per cent over toria and brown sarson, (as indicated by the average values of two years for both crops) but in the middle and concluding stages of blooming the excess is about 60 per cent (Table II).

(5) In the stem and branches the essential oil is at its maximum in the early stage of plant growth. Later on from the blooming stages onwards, however, considerable decrease in the essential oil content takes place. When the plants are very young, the differences between the various crops under study are imperceptible, but, with the advancement in ages, the differences become manifest. Higher amounts of essential oil persist in raya varieties in later stages of growth than in toria and brown sarson (Table II).

It may be pointed out that in the two years there are slight variations in the essential oil content in the developing ovules and in other parts. These variations may be attributed to errors in sampling or they may be seasonal varia-These, however, are of little consequence, since the general conclusions are based on two years' data, which show the same trend.

From the results of the essential oil content in various parts in these crops it is therefore concluded that the greater pungency in raya and taramira crops appears to be due to the presence of higher amounts of essential oil in the developing seeds as compared with those of toria and brown sarson crops. In the case of leaves, stem and branches this difference is less marked although the former crops tend to possess a higher amount of essential oil in these parts, particularly in leaves in the later stages of growth. Brown sarson having the least amount of essential oil is evidently more suited for fodder purposes than the other species.

#### ACKNOWLEDGEMENTS

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# INFLUENCE OF AGRONOMIC TREATMENTS ON GINNING PERCENTAGE IN PUNJAB COTTONS

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Among the various economic attributes of the cotton plant, high yield, high ginning percentage and good quality of lint are evidently the most important. Of these, yield always takes precedence over others and in all centres where work on cotton improvement is being carried out, the consideration of yield has always ranked the foremost. The importance of this character has been fully brought out by Panse [1941] who finds that, to compensate for reduction in yield by 1 per cent, the variety must show an increase of 10 per cent in quality. As regards ginning percentage and quality, it is the experience of most cotton breeders that these two attributes are antagonistic and sometimes improvement in quality has been achieved at the cost of ginning percentage. It is, however, noteworthy that a loss of 1 per cent in ginning per-centage eats up nearly 3 per cent of the price of the produce, which means that 1 per cent ginning outturn is equal to 3 per cent yield of the crop or 30 per cent in quality. In view of this, it is necessary that, in any programme of cotton improvement, ginning percentage must receive at least as great a weightage as the yield of the crop or the quality of its produce.

The primary characters which contribute to ginning percentage are the weight of lint and weight of seed, each of which is a complex character by itself, consisting of several subcomponents. For instance, the weight of lint is composed of weight of single fibre and the number of fibres on a seed. The former character could be further split up into still finer components, such as fibre length and fibre weight per unit length. Similarly the important sub-components of seed weight are volume and the specific gravity of seed

All the above mentioned characters are heritable but like all quantitative characters, each of them is subject to great variations under the stress of seasonal and agronomic factors also. The work of Balls [1912] in Egypt, Sturkie [1934] and Brown [1938] in America, and several others, has clearly revealed the great role played by nutrition, soil moisture, spacing, soil types, climate, etc., in modifying ginning percentage and its components. In India, however, such analysis has received but little attention from cotton workers, and the exact nature of factors, which

percentage of varieties, is still not clearly understood. The only results reported on Indian cottons are those of Patel and Mankad [1923], Venkataraman [1930] and Sen [1934], who have studied the variations in some of the components of ginning percentage in progressive pickings of varieties, but no worker has so far attempted to analyse the bearing of the more commonly used agronomic treatments on this important character. The present investigation was undertaken with a view to filling up the latter gap in the knowledge about Punjab cottons, and an attempt has been made to study the variation in the ginning percentage of some desi and American varieties when they are grown under different irrigational, manurial, sowing date and spacing treatments. The results obtained in this study are embodied in the present

#### MATERIAL AND METHODS

The data for studying the effect of different irrigational and manurial treatments were gathered from the following experiments conducted by the Deputy Director of Agriculture, Lyallpur, during 1942-43 and 1943-44 on the Punjab-American cotton variety L.S.S. under the Water Requirement of Crops Scheme, Risalewala:

(i) Different number of irrigation experiments.

(ii) Different dates of irrigation experiments.(iii) Experiments to study the effect of different

intensities and frequencies of irrigation.

(iv) Flat versus ridge irrigation experiments.

(v) Manurial-cum-irrigational experiments.

All these experiments were carried out in accordance with the randomized block system with five to six replications.

The quantity of water applied at each irrigation was measured by first pumping the calculated quantity of water into a high level cistern and then letting out the water through cement concrete pipes into the beds. In this way, measured quantities of water were given to each bed without any loss.

The samples for appraising the effect of sowing dates and spacings were taken from two different complex experiments laid out during 1942-43 at the Cotton Research Farm, Risalewala. The details of the treatments included in these experiments are given later. Their layout was according to the split plot design.

The samples for determination of ginning percentage consisted of 3 lb. kapas taken separately from each of the replications in the above experiments.

Ginning of all samples was done by manual labour with wooden hand gins. Before ginning, the samples of kapas were conditioned for 24 hours in an air tight room artificially kept at a constant temperature (90°F.) and humidity (30 per cent). The lint obtained after ginning was also treated similarly before weighing it finally for the calculation of ginning percentage.

For determining the various components of ginning percentage, the following procedure was adopted:

(a) Seed weight. The combined seeds of all the replications were divided into 16 equal parts. Twenty-five seeds from each lot were then selected at random and mixed to form a representative sample of 400 seeds. This sample was weighed on a chemical balance and, from the total weight, the average weight of a single seed was calculated in miligrammes.

(b) Lint per seed. This was calculated by the following formula:

Average weight of a single seed × Ginning outturn

100-Ginning outturn
(c) Mean fibre length. This was found out by Ball's Sorter from representative samples drawn out of the mixed produce of all replications of the experiment.

(d) Mean fibre weight. This was calculated by weighing a known number of fibres on a quartz Torsion Micro-balance.

EXPERIMENTAL RESULTS

(A) Effect of varying quantities of irrigation water. The quantities of irrigation water tried in these experiments ranged from 9 to 24 acre-inches. These were applied in 3 to 8 irrigations, each of 3 in. depth.

The analysis of variance in these experiments revealed the effect due to irrigation as highly significant in both the years. The mean values of ginning percentage along with critical difference and order of merit of different treatments are given in Table I.

Table I

Mean values of ginning percentage in different number of irrigation experiments

			Number o	f irrigations			Critical difference	Order of merit
Year	3 ″	4	5	6	7	8	(5 per cent)	order of merit
1942–43	33.60	33.97	33.72	33.06	. 33.36	33.36	±0·40	4, 5, 3, 7, 8, 6
1943-44	31 · 25	30.64	30.18	30.00	29.70	29.43	±0.43	3, 4, 5, 6, 7, 8

The results show that, in 1942-43, four irrigations gave significantly higher ginning percentage than six, seven and eight irrigations, though it did not vary significantly from three and five irrigations. In the year 1943-44, there was a regular decline in ginning percentage as the quantity of water applied to the crop increased, 3 and 4 irrigations giving significantly higher values for this character than all the other treatments. The important conclusion, therefore, to be drawn from the combined results of the two years is that the ginning percentage is likely to increase under short water supply and decline with heavier quantities of water.

In order to find out the reasons for the increase of ginning percentage under reduced water supply

and its decline with more liberal irrigations, an effort was made to split the ginning percentage into its important components, namely, seed weight, lint weight per seed, mean fibre length and mean fibre weight. The results of this analysis are given in Table II. It may be mentioned that, since all the constants other than the ginning percentage were determined from pooled produce of all the replications in the experiment, it was not possible to find out the true standard error of those constants. Consequently in Table II, no figures relating to critical differences have been given. This, however, does not reduce the value of the results, as the differential effect of various treatments is quite marked.

Table II

Mean values of ginning percentage and its components in the different number of irrigation experiments

1	lues of granting percentage		N	umber of irrig	ations		
Year	Characters	3	4	5	6	7	8
1942-43	Ginning percentage Seed Weight (mg.) Lint weight per seed (mg.) Fibre length (in.) Fibre weight per unit length (10-6 gm./cm.)	33·60 60·58 30·34 0·885 3·44	33·97 61·15 31·16 0·907 3·51	33·72 65·20 33·33 0·915 3·62	33·06 67·55 33·35 0·915 3·66	33·36 69·72 34·80 0·915 3·91	33·36 70·95 35·51 0·927 4·03
1943-44	Ginning percentage Seed weight (mg.) Lint weight per seed (mg.) Fibre length (in.) Fibre weight per unit length (10 <sup>-5</sup> gm./cm.)	31·25 65·40 30·38 0·88 3·54	30:64 65:90 29:75 0:91 3:77	30·18 69·60 30·56 0·91 3·78	30·00 70·00 29·95 0·91 3·79	29·70 73·33 30·82 0·92 3·83	29·43 74·50 30·54 0·93 3·86

It will be observed that the three characters, seed weight, fibre length and fibre weight, showed a progressive increase with an increase in the number of irrigations in both the years of the experiment, while the behaviour of lint weight per seed varied considerably within the two years. In the year 1942-43, this character, like seed weight, fibre length and fibre weight, maintained an upward trend with the increase in number of irrigations, while in the subsequent year (1943-44), it remained more or less at a constant level in all the irrigations. This observation is in harmony with that of Patel and Mankad [1923] who also observed the lint weight per seed to be a more variable character than seed weight: The ultimate effect of the increase in lint weight per seed during 1942-43 was to partially out-balance the reducing effect on ginning percentage of increased seed weight, so that the ginning percentage in case of lower to higher number of irrigations within this year varied only very slightly. On the other hand, the constancy of lint weight per seed in the year 1943-44 allowed increased seed weight to have its full play, with the result that the drop in ginning percentage due to increase in number of irrigations was more marked during this year than that in 1942-43.

Still another significant feature which requires special emphasis in connection with the above

observations is that although fibre length and fibre weight under 8 irrigations were significantly higher than that of 3 irrigations during 1943-44, yet the lint weight per seed within these extremes of irrigations exhibited no appreciable differences. The logical conclusion of this is that fibre length and fibre weight form but very unimportant subcomponents of lint weight per seed. In the determination of the latter character, the primary place, perhaps, goes to number of fibres per seed, as has also been inferred by Leake [1914], and it will be of great interest to study the behaviour of this component under differential irrigation treatments.

(B) Effect of different frequencies and intensities of irrigation

In experiments designed to study this aspect, a total of 20 acre-inches of water was applied to the Punjab-American variety L.S.S. in 3, 4, 6, 8 and 9 irrigations of equal intensities in the year 1942-43, and 3, 4, 5, 6 and 8 irrigations in the year 1943-44.

The analysis of variance revealed non-significant effect of treatments in the first year of the experiments and significant in the second year. The mean values of ginning percentage obtained in both these years along with critical differences and order of merit of different treatments is shown in Table III.

TABLE III

Mean values of ginning percentage under different intensities and frequencies of irrigation

	<b>I</b>	2	3	4	5	0		
Year	3 . Irrigations each of 6.7 in.	4 Irrigations each of 5 in.	5 Irrigations each of 4 in.	6 Irrigations each of 3.3 in.	8 Irrigations each of 2.5 in.	9 Irrigations each of 2·2 in.	Critical difference (5 per cent)	Order of merit
1942-43	33.71	34.04		34.04	34 · 32	34 · 24	Non-signi- ficant	5, 3, 4, 1, 2
1943-44	28.47	28.34	28.60	28.56	30.21		∓0.80	0, 0, 4, 1, 4

It will be seen from Table III that the values of ginning percentage remained at an almost constant level in different treatments during 1942-43. In 1943-44 also there were no differences in the ginning percentage of the crop receiving 3. 4. 5 and 6 irrigations of varying intensities, but, during this year, the application of 8 irrigations, each of 2.5 in. depth, produced significantly higher ginning percentage than all the other treatments.

(C) Effect of irrigating the crop on different dates In these experiments, a total of 20 in. water was applied in six irrigations as follows:

(i) Early start and early finish of irrigations, the dates of watering being 27/6, 21/7, 16/8, 5/9, 21/9 and 3/10. The depth of each irrigation was 3:33 in.

(ii) Medium start and medium finish, the dates of application of irrigations being 17/7, 16/8, 5/9, 21/9, 3/10 and 15/10, and the depth the same as in (i).

(iii) Late start of irrigation and late finish, viz. on 29/7, 31/8, 5/9, 1/10, 15/10 and 29/10. The depth of each irrigation in this case was also

(iv) As in (i), but the earlier three irrigations were lighter (2.5 in. each) and the later three

heavy (4.17 in. each).

The experiments were performed only for one year (1942-43) and the analysis of variance showed the effect of the treatments to be non-significant. The mean values of ginning percentage were 34.21, 34.32, 34.26 and 34.11 for treatments (i), (ii), (iii) and (iv) respectively, from which it can be inferred that, so long as the total quantity of irrigation water remained the same, starting the irrigations to the crop early or delaying them by even one month further, did not make any difference in

the ginning percentage of varieties.

Another noteworthy observation in this experiment is that even treatment (iv), in which apart from time of irrigation, the intensity of waterings was also varied, did not exhibit any different ginning percentage from other treatments. This observation fully corroborates the conclusion set forth earlier in this paper that, within a fixed quantity of irrigation water, the variations in the intensity of irrigations have no appreciable effect on ginning percentage. The results also justify the conclusion that no increase in ginning per-centage of varieties is likely to result from application of lighter irrigations in the earlier stages of the growth of the crop, and heavier ones at the supposedly critical time of development of lint fibres.

(D) Flat versus ridge irrigation

Study of this irrigational phase was also done

only for one year, viz. 1942-43. The sowing of the crop was done on flat, and the first irrigation was also applied in the ordinary way, but subsequently ridging was done in those plots where ridge irrigation was contemplated. The total quantity of water applied to the ridged and the flat plots was the same, viz. 16 in.

The analysis of variance revealed the effect of treatments to be non-significant, the mean values of ginning percentage under flat and ridge irrigations being 34.18 and 34.43 respectively. These results show that the method of application of water is not likely to make any difference in the ginning percentage of varieties, provided the total quantity of water remains the same.

(E) Effect of fertilizers

The data for this study was obtained from the manurial-cum-irrigational experiments of the Water Requirement of Crops Scheme, Risalewala, on L.S.S. The experiments were carried out only for one year (1943-44) and included the following treatments:

(i) Manures. No manure (M<sub>0</sub>) and 40 (M<sub>1</sub>), 80 (M<sub>2</sub>) and 120 (M<sub>3</sub>) lb. of nitrogen per acre from

sodium nitrate.

(ii) Irrigations. Four irrigations (W1), 6 irrigations (W2) and 8 irrigations (W3) with 3 in.

depth of water in each case.

The analysis of variance showed significant effects of both fertilizers and irrigations individually, though their interaction was non-significant. The mean values of ginning percentage obtained in case of each of these treatments are given in Table IV.

TABLE IV

Mean values of ginning percentage in manurialcum-irrigational experiment

			rtilizer gation		Critical differ-	
Treatment	Mo	$\frac{M_1}{\overline{W_1}}$	M <sub>2</sub> W <sub>2</sub>	$\frac{M_3}{W_3}$	ence (5 per cent)	Order of merit
Manures Irrigations	30·69	30·11	29·42 29·67	28·96 29·52	±0.34 ±0.30	$M_0, M_1, M_2, M_3 W_1, W_2, W_3$

The results show that the ginning percentage declined progressively as the dose of the fertilizer increased. This tendency was so marked that all the doses varied significantly from each other. A similar decline also occurred with an increase in the number of irrigations, though from statistical stand-point only four irrigations gave significantly higher ginning percentage than the other two irrigational treatments, which did not vary inter se. These results fully confirm those given

earlier in this paper regarding the effect of varying quantities of water on ginning percentage.

Of still greater interest than the above result is the variation produced in different components of ginning percentage by different manurial treatments. The figures obtained with such treatments are presented in Table V.

Table V

Average value of ginning percentage and its components for different doses of sodium nitrate

		Doses of f	ertilizer	
Characters	Mo	M <sub>1</sub>	M <sub>2</sub>	Мз
Ginning percentage Average seed weight	30·68 71·28	29·99 73·58	29·42 75·11	28·96 78·46
(mg.) Average lint weight per seed (mg.)	31.6	31.59	31 · 33	32.01
Mean fibre length (in.)	0.890	0.903	0.908	0.916
Mean fibre weight per unit length (10-6 gm./cm.)	3.46	3.64	3.81	3.98

The data show that, with an increase in the dose of the fertilizer, a progressive increase occurred in seed weight, fibre length and fibre weight. It would, however, be noticed that the increase in the latter two characters was very small indeed, so that their contribution towards increasing the average weight of lint per seed was almost negligible. The net result, therefore, was that the seed weight under different doses of fertilizer increased tremendously, while the lint weight per seed remained almost constant. Thus, the main factor causing reduction in the ginning percentage in this case was the seed weight.

(F) Effect of sowing dates and spacings

The data for this study were collected from two different complex experiments laid at the Cotton Research Farm, Risalewala, during 1943-44. The first of these included the following treatments:

(i) Varieties. 39 Mollisoni, L.S.S. and 199F. (ii) Spacings between rows. 1 ft. (Sp<sub>1</sub>), 2 ft. (Sp<sub>2</sub>), 3 ft. (Sp<sub>3</sub>).

(iii) Sowing dates. 6/5 (SD<sub>1</sub>), 28/5 (SD<sub>2</sub>), 19/6

 $(SD_3)$ .

The analysis of variance in these experiments showed the effect of sowing dates to be highly significant in American varieties (L.S.S. and 199F) but not in desi (39 Mollisoni). In contrast to this, the effect of spacings was uniformly significant in both desi and American varieties. The interaction between spacings and sowing dates, however, was non-significant in all the types.

The mean values of ginning percentage with

different sowing dates and spacings in each of the three varieties are given in Tables VI and VII respectively.

Table VI

Mean values of ginning percentage with different sowing dates

Varieties	SD <sub>1</sub>	$SD_2$	SD <sub>3</sub>	Critical differ- ence (5 per cent)	Order of merit
39 Mollisoni L.S.S. 199F	32.23	34·00 31·08 36·64	33·66 28·98 36·91	N.S. ±0.876 ±0.627	$SD_1$ , $SD_2$ , $SD_3$ $SD_1$ , $SD_3$ , $SD_2$

Table VII

Mean values of ginning percentage with different spacings

Varieties	Sp,	Sp <sub>2</sub>	Sp3	Critical differ- ence (5 per cent)	Order of merit
39 Mollisoni L.S.S.	34·90 31·27		32·74 30·29	±0.699	Sp <sub>1</sub> , Sp <sub>2</sub> , Sp <sub>3</sub> Sp <sub>1</sub> , Sp <sub>2</sub> , Sp <sub>3</sub>
199F	37.63	37.30	36.54	士0.519	$\underline{\operatorname{Sp_1},\operatorname{Sp_2},\operatorname{Sp_3}}$

The data presented in Table VI show that, in the American variety L.S.S. the results with all the sowing dates varied significantly from one another, first sowing date giving the highest value and third the least. In case of variety 199F, first sowing date gave significantly better ginning percentage than the second and the third, but the difference between the latter two was non-significant.

As regards spacings, its effect was most marked in the desi variety, 39 Mollisoni, where the results with each of the spacings tried varied significantly from one another, highest ginning percentage having resulted from 1 ft. spacing and the least from 3 ft. spacing between rows of the crop. In both the American varieties also, the effect of spacings was similar to that in 39 Mollisoni, the closest spacing yielding highest value of ginning percentage and the widest the least as will be noticed from Table VII, but the differences were not as distinctly significant as in the case of Mollisoni.

The second set of experiments, from which data for studying the effect of spacing and sowing dates on ginning percentage were obtained, included the following treatments:

(i) Spacings between rows and plants:  $1 \text{ ft.} \times 1 \text{ ft. } (Sp_1); 2 \text{ ft.} \times 1 \text{ ft. } (Sp_2); 3 \text{ ft.} \times 1 \text{ ft. } (Sp_3); 2 \text{ ft.} \times 2 \text{ ft. } (Sp_4); 3 \text{ ft.} \times 2 \text{ ft. } (Sp_5).$ 

(ii) Sowing dates. 16/5 (SD<sub>1</sub>), 4/6 (SD<sub>2</sub>), 26/6 (SD<sub>3</sub>).

Only one variety was used in the experiment, viz. the Punjab-American cotton, 4F.

The mean values of ginning percentage obtained in each of the above mentioned treatments are given below:

These results are in close conformity with those of previous experiments and show conclusively that the ginning percentage of the variety 4F was depressed as sowings were delayed and also as the spacings between rows and plants became wider.

#### Discussion

The bearing of some of the important agronomic treatments on ginning outturn of a few Punjab desi and American varieties has been given in the foregoing pages. The results show that flat versus ridge irrigation did not influence ginning percentage to any appreciable extent. Similarly, in the experiment designed to find out the effect of irrigating the crop on different dates, no significant differences were observed in the ginning percentage of varieties due to types of irrigation employed. The results of intensities and frequencies of irrigation experiments, however, reveal that 20 acre-in. of water, when applied in 8 equal irrigations gave significantly higher ginning percentage in 1943-44, but that the same treatment failed to produce any marked effect on ginning outturn in 1942-43. Since the other frequencies and intensities of irrigation in both the years did not show any significant differences inter se, and since even 8 irrigations gave very inconsistent results in the two years, it is safe to conclude that the frequency or intensity of irrigation also has no effect on ginning percentage, so long as the total quantity of irrigation water applied to the crop remains the

In contrast, however, to the above treatments, the application of different quantities of water to the variety L.S.S. has given consistent results in both the years of experimentation, the ginning percentage in general increasing under short water supply and vice versa. Thus, from a practical

stand point, it is the total quantity of water applied to the crop which is really effective in modifying the ginning percentage of varieties and not its intensity, or date and method of application. The relevent question, however, to be asked in this connection is whether the application of lower quantities of water could be advocated as a method of obtaining higher ginning percentage of cotton varieties? The answer to this question can be given by considering the variations that are produced in different components of ginning percentage as a result of the application of different quantities of water. The present investigation has shown that the seed weight goes down with the reduction in the quantity of irrigation water, which means that a greater percentage of immature seeds is likely to result from such a treatment. Since such undeveloped seeds cannot be expected to bear lint of proper maturity and length, it is doubtful if reduction in the quantity of irrigation water will ever be a practical proposition for achieving higher ginning percentage. This conclusion is supported by the results of Sturkie [1934], who found an increase in ginning percentage with decrease in water supply and concluded that this increase was due to a greater decrease in seed weight than to any corresponding decrease in lint weight per seed.

The effect of the addition of increasing doses of inorganic manure, sodium nitrate, has also been to depress ginning percentage. This result is in agreement with that of Brown [1928] who states that cotton grown on rich lands or land with liberal application of sodium nitrate has lower ginning percentage than cotton grown on less fertile soils. The analysis of ginning percentage into its constitutent components in the manurial experiment further reveals that the depression in this character as a result of increasing dose of the fertilizer is due mainly to increase in seed weight, to which extent the addition of inorganic manures is desirable from a practical stand point for reasons similar to those given for application of heavier

doses of irrigation water.

As regards the effect of spacings on ginning percentage, it is evident from the results that closer spacing (i.e. 1 ft.×1 ft.) led to significantly higher values of this character than the medium or wider spacings. This is probably due to the greater number of plants per unit area in closer spacings and thus to smaller amount of nutrient matter available to each plant for normal development of seed and lint. It would be of great interest to study the variation in seed weight and lint weight per seed of plants grown with different spacings to throw further light on this aspect of the problem. The work of Balls [1912] reveals that lower ginning outturn with wider spacings

is due to greater increase in seed weight than to any corresponding increase in lint weight per seed.

The effect of sowing dates on ginning outturn has also been quite marked in that the first sowing date has given higher ginning outturn than the other sowing dates in case of Punjab-American varieties only. Although it has not been found by actual studies how this variation in ginning outturn of different sowing dates is brought about, yet it is probable that seed weight is low in the crop yielded by the first sowing date, which would naturally push up its ginning outturn. But, as has been mentioned above a low seed weight is likely to be accompanied by lint of poor quality, it may not be desirable from a practical stand point to advocate early sowings of cotton. Further justification for this conclusion is afforded by the fact that the yield and quality of the crop in later sowings show a marked improvement over the early sowings in the Punjab, as has been established by the work of Trought [1930] and Dastur and Mukhtar Singh [1942].

The above discussion amplifies the fact that the scope for achieving higher ginning percentage of varieties, at least in the Punjab, by effecting changes in the commonly used agronomic treatments is very meagre indeed. The limit to such a possibility is set by the seed weight, which has been seen to be affected by these agronomic treatments to a much greater degree than the lint weight per seed. This fact doubly increases the responsibility of the cotton breeder on whom the task of maintaining ginning percentage, in the new varieties evolved by him, at the highest possible pitch becomes incumbent, so that those varieties even after being subjected to liberal water supply, manuring, late sowings, etc. which are essential for obtaining proper yield and quality, can still give a ginning percentage not below the economic level.

SUMMARY

The variations produced in the ginning percentage of a few Punjab-American and desi varieties of cotton by some of the important agronomic treatments have been studied.

By application of 3 to 8 irrigations, each of 3 inches depth, the ginning percentage generally declined as the quantity of water applied to the crop increased. This reduction is mainly the result of increase in seed weight.

The application of 20 in. water in 3 to 9 irrigations of different intensities failed to affect ginning outturn appreciably during 1942-43, but in the succeeding year 8 irrigations, when applied in doses of  $2\frac{1}{2}$  in. each, gave significantly higher ginning percentage than all the other intensities.

So long as the total quantity of water applied to the crop remained the same, starting irrigations early or delaying them by even one month did not make any difference in the ginning percentage.

Application of lighter irrigations in the early stages of growth and heavier ones near the flowering and fruiting time of the crop produced no change in ginning percentage.

There was no difference in the ginning percentage of crop grown under flat or ridge irrigation.

The effect of addition of increasing doses of sodium nitrate was to progressively depress the ginning percentage mainly as a result of increase in seed weight.

Sowing dates and spacings affected the ginning percentage of varieties significantly, higher values of this character having been obtained with early sowings and closer spacings.

The possibility of achieving higher ginning percentage of varieties by effecting changes in the commonly used agronomic treatments in the Punjab has been discussed.

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# INVESTIGATIONS ON SOME FUNDAMENTAL ASPECTS OF CITRUS PROPAGATION IN ASSAM

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The genus Citrus comprises a wide range of fruits which are prominent in both the internal and external trade of Assam. Some of the species, namely the Khasi orange (C. chrysocarpa Lush.), lemon (C. limonia Osbeck) and lime (C. aurantifolia Swingle) occupy a total of about 25,000 acres and are commercially the most important. Besides, there are other species like shaddock (C. maxima Merril), citron (C. medica Linn.), C. macroptera Mont. and C. limettioides Tanaka, to name a few, which though commercially unimportant, are nevertheless grown in many a homestead for family needs. Notwithstanding the antiquity and the economic importance of citrus cultivation, the province had, hitherto, taken no serious interest in guiding the industry on a sound scientific basis. Left entirely to private initiative and enterprise it began to develop by feeling its way according to its own light. Technical methods so widely and advantageously adopted in other parts of the world did not seem to have much influence here. Propagation, as in nature, was carried on almost entirely through seeds. The only man-made device was 'gootee' or 'marcotte' a process which seems to have been independently developed and employed for the propagation of those varieties of limes and lemons which Nature most unkindly did not provide with numerous seeds for easy and quick multiplication.

With the inauguration of the citrus research at Burnihat, Assam, under the joint auspices of the Provincial Government and the Imperial Council of Agricultural Research, India, attempts are now being made to make up for the time lost and evolve techniques of propagation and cultivation for developing the industry on a sound and a rational basis. This paper deals with some of the fundamental aspects of propagation in the nursery.

#### Polyembryony in different citrus varieties

Polyembryony or the presence of more than one embryo within a seed, has long been known to occur in certain varieties of citrus [Leeuwenhoek, 1719; Frost, 1926; Toxopeus, 1931; Webber, 1937 and Naik, 1939]. The importance of this phenomenon in a nursery lies in the fact that once the gametic seedlings which are generally weak

growers are eliminated the remaining seedlings of apogamic origin conform to the parental type and thus it becomes possible to establish clonal lines for a variety in a most natural way without taking recourse to any artificial means. With a view to observing to what extent this phenomenon occurs in some of the important indigenous citrus varieties, a preliminary study was carried out in the seedbed, counting separately the seeds producing one seedling each and those producing more than one.

Table I

Percentage of polyembryonic seeds in different citrus varieties detected at germination

Number of minated s examined	Two	Three		
1	seed- lings	seed- lings	Four seed- lings	Percent of seeds producing more than one seed-ling
380	138		2	36.84
	Martin L			
146	94			24.64
140	9≄	2	••	24.04
	-			
200	66	1		33.50
1344 2				
252	79	4		32.93
	Maria de la composición del composición de la composición de la composición del composición de la composición del composición de la composición de la composición del compos			
000				10.41
328	44	••		13.41
		100		
469	122	5		27.08
				- 00
160	24			15.00
496	••			nil
190				
130		••	••	nil
	146 200 252 328 469	146     34       200     66       252     79       328     44       469     122       160     24       496	146     34     2       200     66     1       252     79     4       328     44        469     122     5       160     24        496	146     34     2        200     66     1        252     79     4        328     44         469     122     5        160     24         496

The foregoing figures are at best a rough indication of the extent of polyembryony present in these varieties, for it is not sure that all seeds producing one seedling each are monoembryonic. Polyembryonic seeds of citrus usually seem to produce not more than two seedlings each. Further

the total absence of polyembryony in shaddocks and citrons tends to show that the phenomenon is not common in all the citrus species. This is in conformity with the observations of Naik [1940]. The extent of polyembryony in the Khasi orange (C. chrysocarpa Lush.) is about 27 per cent and this probably explains why in spite of continuous natural cross pollination, there is such a comparative great uniformity of seedling trees as is found in the province.

GERMINATION OF ORANGE SEEDS (C. CHRYSOCARPA LUSHINGTON) AT DIFFERENT STAGES OF MATURITY OF FRUITS

As has been stated before, the propagation of loose-skinned oranges in Assam is carried on almost exclusively through seeds. An experiment was therefore conducted with the object of ascertaining the stage of maturity of fruits at which the maximum germination may be obtained. Twentyfive fruits were picked from a particular orange tree once every fortnight and 200 well-developed seeds selected from the fruits of each lot were put, immediately after extraction, in a sandbed for germination.

TABLE II

Percentage of germination of seeds at different stages of maturity of fruits, the percentage of polyembryonic seeds among them and the time taken for germination to start and its completion

Dates of sowing	Number of seeds germinated	commen- cement of germi- nation in days	for com- plete germi- nation			age of Polyem- bryonic seeds detected
15.9.41	95	26	42	47.5	20	21.05
1.10.41	82	31	47	41	19	23 · 2
15.10.41	128	26	65	64	30	23 · 4
1.11.41	175	33	60	87 · 5	36	20.6
15.11.41	190	31	65	95	39	20.5
1.12.41	186	30	67	93	41	22.0
15.12.41	190	31	65	95	50	26.3
1.1.42	174	35	68	87	37	21.2
15.1.42	166	37	72	83	37	22 · 2
1.2.42	162	45	76	81	43	26.5

The mean outside maximum temperature from September, 1941, to May, 1942, month by month was 89.3°, 86.5°, 77.2°, 74.2°, 71.0°, 76.5°, 81.8°, 85.5° and 87.2°F. respectively. It will be seen that inspite of a gradual rise in temperature from February onwards, the time required for germination for the connected sowings has increased and the percentage of germination for the same has

decreased to a certain extent, though it is known that rise in temperature hastens germination. So the differences in the percentage of germination and the time required for germination in this case are probably due to differences in maturity of fruits. The results indicate that the seeds of mature fruits have a better viability than those of less mature ones. Over-ripening of fruits appears to lower the germinating capacity of seeds to a small extent and prolongs the period of germination quite considerably. The interval between the time of sowing and the commencement of germination seems to increase with the advancing maturity of fruits.

Immaturity of seeds has probably a more detrimental effect on the gametic rather than on the nucellar embryo as is evident from the fact that irrespective of the stages of maturity of fruits, the percentage of germination of polyembryonic seeds is fairly constant ranging from 20.5 to 26.5. Perhaps the nucellar embryos develop sooner than the gametic ones in this variety.

EFFECT OF SUNNING ON THE GERMINATING CAPACITY OF ORANGE SEEDS (C. CHRYSUCARPA LUSHINGTON)

The popular belief among the growers is that to ensure satisfactory germination the seeds of all kinds should be as thoroughly dried as possible by subjecting them to direct sunshine for protracted periods. In order to see how far this rule is applicable to the orange a small quantity of orange seeds from mature and healthy fruits from a single tree was subjected to different periods of sunning from zero to forty hours and tested for germination in a sandbed.

Table III

Percentage of germination of orange seeds
subjected to different periods of sunning

Date of sowing	Duration of sunning in hours	Number of seeds sown	Number of seeds germi- nated	Percentage of germination	Time taken for commencement of germination in days
26.1.42	0	200	192	96	33
26.1.42	4	,,	162	81	33
27.1.42	8	,,	102	51	36
28.1.42	12	,,,	62	31	38
29.1.42	16	,,	12	6	41
30.1.42	20	,, ,, ,,	162 102 62 12 14 6	6 7 3	39 54 49
31.1.42	24	,,	6	3	54
1.2.42	28		2	1	49
2.2.42	32	" "	2 2	1	50
3.2.42	4 8 12 16 20 24 28 32 36 40	,,	nil	nil	1 4
4.2.42	40	,,	nil	nil	

The foregoing results indicate that the germinating capacity of orange seeds progressively deteriorates with increased doses of sunning, that the fresh seeds ensure the highest germination and that in no case should the seeds be exposed to direct sun for more than four hours. The time taken for commencement of germination seems to increase with the increased period of sunning.

### DURATION OF THE VIABILITY OF CITRUS SEEDS

The object of the experiment was to find out how long the viability of citrus seeds remains unimpaired if these are kept in an airtight container. The significance of this experiment lies in the fact that sometimes it becomes essential to send the seeds to a distant place or to preserve them for deferred sowing. Seeds of seven varieties of citrus representing three grades, viz. big, medium, and small, were selected for the experiment. The seeds of different varieties were extracted from well-matured fruits on 29 December, 1943, and dried in shade for three days. Four hundred seeds of each variety mixed with charcoal powder were kept separately in a desiccator for two and a half months after which one hundred seeds of each variety were put in a sandbed for germination. The remaining seeds were sown at intervals of seven days from the date of first sowing.

The results tend to show that the big sized seeds retain viability for a longer period than the small ones and that there is a gradual fall in vitality of stored seeds irrespective of size.

Table IV

Percentage of germination, size of seeds, and minimum time required for germination to commence

Name of variety of seeds	$\begin{array}{c} \text{Number of} \\ \text{seeds sown} \end{array} \qquad \begin{array}{c} \text{Average size of seeds} \\ \text{(length} \times \text{breadth)} \end{array}$		Date of sowing	Number germinated or per cent of germination	Time interval for germina- tion to start (in days)
: 11명 (2 - 2 - 2 - 2 - 2 - 2 - 2 - 2 - 2 - 2		2.17 22 20	20.3.44	78	44
Rabab Tenga (C. maxima	100	$1.94 \text{ cm.} \times 1.12 \text{ cm.} = 2.17 \text{ sq. cm.}$	27.3.44	64	39
Merril)	,,		3.4.44	30	40
영, 문화 호텔 일일 보이었는다고 있다면 있는 말하다	,,		10.4.44	15	31
	"				
T I (C) annualism	100	$1.82 \text{ cm.} \times 0.82 \text{ cm.} = 1.49 \text{ sq. cm.}$	20.3.44	56	42
Karun Jamir (C. aurantium			27.3.44	40	38
Linn.)	"	[1] : [1] [1] [2] [2] [2] [2] [2] [2] [2] [2] [2] [2	3.4.44	26	43
[발생하고 내시가 뭐 되는데 보다.	)) ))	[[] [ [ [ [ [ [ [ [ [ [ [ [ [ [ [ [ [ [	10.4.44	10	38
			20.3.44	48	41
Muri Tenga (C. limettioides	100	$1.52 \text{ cm.} \times 0.80 \text{ cm.} = 1.02 \text{ sq. cm.}$	$\frac{20.3.44}{27.3.44}$	32	38
Tanaka)	2,9		3.4.44	14	42
사고 많아내다 그 경우에 어디를 하는	,,		10.4.44	nil	
	. ,,		10.1.12		
	100	$1.32 \text{ cm.} \times 0.68 \text{ cm.} = 0.89 \text{ sq. cm.}$	20.3.44	52	39
Jora Tenga (C. medica Linn.)		1 92 om. x 0 60 cm.	27.3.44	46	40
	,,	세계 : [1] [1] [1] [1] [1] [1] [1] [1] [1] [1]	3.4.44	32	34
	22		10.4.44	8	38
	"	내가 많은 없다 말라고 살아 모든 말다면 하다	00 0 44	46	38
Soh Sarkar (C. Karna Raf.)	100	$1.14 \text{ cm.} \times 0.72 \text{ cm.} = 0.82 \text{ sq. cm.}$	20.3.44	36	38
	,,,	기능하다 삼절하다 하다. 그 작은 방송 하는 하는 이렇게	$27.3.44 \\ 3.4.44$	28	37
집에 사용하는 어린다는 얼마나 하게 되었다. 그 이어	,,		10.4.44	nil	nil
어디 내가 아내를 잃다 내는 나를 다	,,,	나의 나를 잘 되는 것이 얼마를 다 먹는다.	10.4.44	7000	
이 동물이 하면 하는 것은 것이 없다.	700	$1.20 \text{ cm.} \times 0.59 \text{ cm.} = 0.60 \text{ sq. cm.}$	20.3.44	32	40
Tulia Tenga (C. limonia Osbeck)	100	1.20 cm. × 0.39 cm. = 0 00 sq. cm.	27.3.44	34	38
되었습니다. 경기 회문에 가는 경기를 하는데	,,,		3.4.44	18	36
	"		10.4.44	14	. 38
	"				40
G. I. Wandong (O limomia	100	$1.24 \text{ cm.} \times 0.45 \text{ cm.} = 0.65 \text{ sq. cm.}$	20.3.44	42	42
Soh Myndong (C. limomia	And the second of	게이의 이 192점 글로그램 방향이 있다면 하시네	27.3.44	36	42 36
Osbeck)		네글로 하고 있다. 하면 있다는 이번 싫었다. 난	3.4.44	33	nil
: BUNG, IB UNIVERSITE (BUNG)	,,	세계 되면 교통되지 않는데 등을 하다.	10.4.44	damaged	7014

Klimenko [1937] has also observed that the vitality of citrus seeds progressively decreases with increased storage.

# Propagation of plants by root-cuttings in the open bed

Investigation into the possibilities of raising plants of different citrus varieties from root cuttings, planted in the open-bed, was made mainly in relation to raising standardized clonal materials. Cuttings of two sizes, viz. 4-6 mm. and 2-3 mm. thickness of 8 in. length, obtained from 2-3 year old trees from 6-8 in. below ground level were planted in a sandbed during July, i.e. when the monsoon had well set in.

Table V

Percentage of success obtained in propagating plants from root cuttings of different citrus varieties

Variety		Diameter of roots in mm.	Number of plants propaga- ted	
Jora Tenga	50	4-6	34	68
(C. medica Linn.)	,,	2-3	28	56
Soh Myndong	,,	4-6	27	<b>54</b>
(C. limonia Osbeck)	,,	2–3	6	12
Muri Tenga	,,	4-6	23	46
(C. limettioides Tanaka)	"	2–3	11	22
Khasi orange	,,	4-6		• •
(C. chrysocarpa Lushington)	,,	2–3		
Karun Jamir	,,	4-6		
(C. aurantium Linn.)	,,	2-3	• •	• •
Rabab Tenga	,,	4-6		
(C. maxima Merril)	,,	2-3		
Satkora (C. macroptera Mont.)	,,	4-6 2-3	••	

Observations indicate that only citrons and lemons may be propagated by this method with appreciable success. The degree of success obtained with rough lemon shows that this method may be profitably employed for multiplication of this important stock type so commonly used for budding different scion varieties. Roots of shaddocks, the Seville orange, Mandarin orange and C. macroptera fail to strike out any shoot when planted in the open bed. General observations, however, indicate that if the stock is removed and

the roots are kept slightly exposed in situ or if the roots are severed from the mother plant and the cut ends are slightly raised above ground and left, then varieties like C. chrysocarpa and C. macroptera, which otherwise do not sprout from planted root cuttings, do form shoots from the exposed root-ends. Roots of 4-6 mm. thickness seem to give a higher percentage of success than those of lesser thickness. This is because root bits of small girth dry up quickly and perish. Halma [1931] and Hunter [1932] also carried out similar investigations on root cuttings without any striking success.

## Propagation from stem cuttings treated with ' Hortomone A '

Of all the different citrus varieties only citrons and lemons may be ordinarily propagated from stem cuttings. An investigation was, therefore, undertaken to ascertain whether it is possible to accelerate root formations in citrons and lemons and induce rootings in other varieties by treating their respective stem cuttings with 'Hortomone A', a synthetic plant hormone or auxin patented by the Imperial Chemical Industries Ltd.

Stem cuttings of seven varieties of citrus were obtained from approximately one and a half year old branches of a particular tree of each. The cuttings were 9 in. in length and 8 to 10 mm. in diameter with a terminal flush of 3 to 4 mature leaves in each [Halma 1931]. Thirty cuttings of each of the varieties cut just below a node and tied in separate bundles, were kept with the lower ends, in a solution of 'Hortomone A' prepared in the proportion of half an ounce to a gallon of water for 16 hours after which the treated cuttings were removed and rinsed thoroughly in water. Thirty cuttings of each variety were left untreated to serve as a control.

The treated cuttings together with a set of controls for each were planted in a slanting manner, two feet apart in a bed of soil mixed with well-rotted cowdung and leaf mould. The planting was done on 6 May 1943, and within three weeks the treated cuttings in most cases began to produce new shoots. The production of new growths was found to vary within the varieties. The cuttings were excavated at the end of three months and the soils attached to the roots were washed away carefully using a pressure sprayer.

TABLE VI

Percentage of survival, mean length of roots, total weights of roots and shoots and ratio of shoot to root of the treated and untreated stem cuttings

Name of variety variety of cut- tings employed in each treament	Number of survival		Per cent of survival		Mean length of root in cm.		Total weight of roots in gm.		Total weight of shoots in gm.		Ratio of Shoot Root		
	Treated	Un- treated control	Treated	Un- treated control	Treated *	Un- treated control	Treated	Un- treated control	Treated	Un- treated control	Treated	Un- treated control	
Jora Tenga	30	28	26	93 · 3	86.6	13.3	10.3	121 · 18	45.08	381 · 6	196 · 2	3.14	432
(C. medica Linn.)  Kata Jamir	,,	28	24	93.3	80.0	10.1	7.4	$56 \cdot 54$	10.34	138 · 14	42.90	2.44	4.14
(C. limonia Osbeck) Soh Myndong	,,	28	26	93.3	86.6	8.1	4.1	$20 \cdot 54$	4.54	46.54	20.84	2.26	8.59
(C. limonia Osbeck) Muri Tenga	,,	16	12	53 · 3	40.0	5.5	2.3	4.70	1.88	27 · 22	9.58	5.79	5.09
(C. limettioides Tanaka) Karun Jamir		18	14	60.0	46.6	3.1	1.06	0.88	0.08	6:40	0.74	7.27	9 25
(C. aurantium Linn.) Rubab Tenga	,,	20	14	66.6	46.6	2.9	0.98	1.64	0.06	6.90	0.64		10.66
(C. maxima Merril) Satkora	,,	18	14	60.0	46.6	0.90	0.47	0.10	Trace	0.20	Trace	2.00	•
(C. macroptera Mont.) Khasi orange (C. chrysocarpa Lush.)	,,,	$m{n}im{l}$	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil

From the above results it may be stated that treatment with 'Hortomone A' causes a higher percentage of survival in cuttings of those varieties which are normally amenable to propagation from cuttings, but it fails to induce rooting in one which ordinarily does not root from cuttings. The chemical, however, seems to have some influence in accelerating both root and shoot formations.

# PROPAGATION OF CITRUS VARIETIES BY 'GOOTEE' OR 'MARCOTTE'

As a method of propagation 'gootee' is probably the most antique and universal in use. All the citrus varieties are amenable to multiplication by this method. And yet for successful nursery practice there is the necessity for collecting informations as to the minimum time required for the formation of roots and the possible percentage of success attainable in different citrus varieties.

Ten varieties of citrus were selected for the study and the 'gootee' operation was carried out in the usual manner [Burns, 1930] during July, i.e. in the height of the monsoon. To find out the actual time of root formations occasional examination was made into the 'gootee' by splitting open the upper sides of the ball of rooting compost. All the varieties were found to form roots within two months and the total rainfall during the corresponding period, i.e. July and August, was 22.60 in. spread over 39 days.

TABLE VII

Range of time required for the formation of roots in the 'gootee' and the percentage of success in different varieties of citrus

Varieties		per of Number success rated		Range of time required for root formation (in days)
Jora Tenga (C. medica Linn.)	2 2 2 2 2 2	20 20 20 20 20 20 20 20 20 20	100 100 100 100 100 100 70 80 90 80	20-25 22-26 31-40 32-40 36-42 38-44 44-52 40-48 45-55 48-54

It may be seen from the foregoing results that all the ten varieties under trial may be successfully propagated by this method if the operation is carried out in the rainy season. There seems to be a distinct varietal difference in the time taken for the formation of roots varying as widely as from 20 to 55 days. Citrons, lemons, C. macroptera and C. limetticides strike out roots much more quickly than the tight-skinned, loose-skinned, Seville oranges and grapefruits.

#### PROPAGATION BY BARK-GRAFTING

In propagating citrus varieties on different rootstocks, budding is by far the commonest and easiest method. But the percentage of success attained by budding during June, July and August is somewhat low due to the prevalence of continuous wet weather and it is in this period that bark-grafting may be adopted in the nursery with

advantage. The operation is akin to crowngrafting with the scion-stick inserted into a slit prepared between the bark and wood of the stock and may be conveniently employed in any stock in which the bark will slip. The small stock plants with a diameter of 0.7 cm. give equally good results as those of a diameter of 2 cm. This method is particularly suitable in inducing an earlier feathering in grapefruits which when budded take an unusually long period ranging from 3-6 months for bud-break. The success or failure of bark-grafting, however, depends very much on the prevailing atmospheric condition. If the operation is followed immediately afterwards by many rainy days the success is assured, but on the other hand if a dry spell lasting even for 4 days intervenes immediately after the operation the percentage of success is much

Table VIII

Effect of dry and wet weather on the success of bark-grafting

Dates of operation .	Number of plants grafted	Number of sunny days in the first week after opera- tion	Number of rainy days in the first fort- night after operation	Number of successful plants	Percentage of success	Mean number of days for bud-break
11 July 1940 16 July 1940 29 July 1940 10 August 1940 22 August-1940 14 September 1940 16 October 1940 18 November 1940	14 20 15 15 20 30 12	1 2 7 7 7	14 15 10 13 7 7	12 16 8 13 7	87 80 53 86-6 35 	38 34 30 42 48 

 $\begin{tabular}{ll} Note : & -Scion-Khasi \ orange \ (C.\ chrysocarpa \ Lushington) \\ & Stock-Soh \ Myndong \ (C.\ limonia \ Osbeck) \\ \end{tabular}$ 

The foregoing results clearly indicate that the optimum season for bark-grafting is during July and August, i.e. during the rains. But even during this period the success depends on the continuity of wet weather. The low percentage of success for the operations carried out on 29 July and 22 August 1940, was due to the fact that in each case the operation was followed immediately afterwards by 4 and 7 sunny days respectively even though there were 10 and 7 rainy days respectively in the later part. The total failure of bark-grafting during the winter months may be attributed to the absence of an active growing condition in the plants and to the attendant low humidity of the atmosphere.

PROPAGATION BY BUDDING
Of the different methods of vegetative propaga-

tion employed in citrus, budding is by far the most efficient and universally practised. There is however, a divergence of opinion among the horticulturists about the details of the technique. The inverted 'T' with a minute piece of wood attached to the bud-shield is advocated by some [Hume, 1941; Wickson, 1926; Camp, 1931; and Naik, 1939] while the removal of the wood from the bud-shield is suggested by others [Hall and Crane, 1933]. In South Africa the question of the retention or removal of wood is considered to be a matter of individual preference [Powell, 1930]. A trial was therefore conducted to find out the relative success in bud-take and joint formation by different methods of budding. The operation was carried out by one single budder using scions of Khasi orange (C. chrysocarpa Lushington) on root-stocks of Kata Jamir (C. limonia Osbeck).

TABLE IX

Percentage of bud-take and the period of bud-break by different methods of budding

Method of budding	Number of plants budded	Mean height in cm. of root- stock	S.E.	Mean stem diameter in mm. of rootstock	S.E.	Number of success- ful bud-take	Percentage of bud-take	Mean period of bud-break in days	S.E.
A. Inverted 'T' with wood	25	65.24	1.28	9.48	0.26	22	88	54.52	1 · 23
B. Inverted 'T' without wood	25	67.24	0.93	9.12	0.27	14	56	56.36	1.56
C. Normal 'T'	25	69.76	1.64	9.40	0.18	18	72	53.61	1.49
with wood D. Normal 'T' without wood	25	69.44	2.59	9.36	0.26	15	60	54.40	1.54

Rootstock- Kata Jamir (C. limonia Osbeck)

Scion— Khasi orange (C. chroysocarpa Lushington) Age of stock plants at budding—11 months

Dates of budding—14.1.42 and 15.1.42

When the various treatments are compared at 5 per cent level of significance, the relation with respect to the various characters are as follows:

Height of stock seedlings— A. B. C. D. Stem diameter of stock-A. B. C. D B. A. D. C. Period of bud-break-

Of the four methods of shield-budding tested both the inverted 'T' and normal 'T' with the wood attached to the bud-shield have given a high percentage of success, viz. 80 per cent and 72 per cent respectively. The former method is customarily practised in this nursery and the slightly higher percentage of bud-take than with the latter may partly be due to a greater skill and dexterity of hand achieved by constant practice. The low bud-take both with normal and inverted 'T' with the wood removed, viz. 60 per cent and 56 per cent respectively is probably primarily due to knife injury unconsciously inflicted on the bud-shield while removing the wood. In any case the removal of the wood does not seem to be justified as the percentage of bud-take is lower and as it unnecessarily taxes the patience, skill and the time of the budder. Besides, the difference of presence or sosence of wood does not seem to have any influence either on the period of budbreak or on the quality of bud-joint. True, the retention of an unnecessarily big or rough woodslice may give rise to a swollen joint [Mendel, 1936] but this only discredits the budder and not the method.

#### INFLUENCE OF SEASON ON THE SUCCESS OF BUDDING

Knowledge regarding the optimum season for budding is essential for successful nursery work. A trial was, therefore, conducted by carrying out budding on a lemon rootstock with bud-wood obtained from a selected orange tree once every month to ascertain the effect of season. All the operations in different months were done by a single budder. In a protracted trial of this nature spread over twelve months of the year the influence

of season, however, gets partially masked by the varying age of the stock materials. In the trial conducted, the age of the stock plants for October operations was only nine months whereas the same for September operation in the following year was about 20 months. The old plants were kept in a workable condition by cutting back the main stem and allowing a shoot to grow on which the subsequent bud-insertions were carried out. This could not be helped as it was not found practicable to maintain a constant age of stock materials by carrying out a monthly sowing primarily because of the short-lived viability of seeds and secondly because of the differential influence of seasons themselves on the growth of the plants.

TABLE X

Success of budding in different months of the year with corresponding period of bud-break

Months		Number of plants budded	Number of success- ful bud- take	Percentage of success	Mean period of bud-break in days
January		25	25	100	55.96
February		,,	21	84	49.90
March		,,	12	48	54.30
April		79	18	72	59.20
May		,,	17	68	49.41
June		,,	15	60	62.20
July		,,	15	60	62.13
August		,,	18	72	56.56
September		,,	20	80	49.90
October		,,	14	56	60.20
November		,,	25	100	57.60
December		,,	25	100	59.50

Rootstock-Kata Jamir (C. limonia Osbeck) Scion —Khasi orange (C. chrysocarpa Lushington)
Seedlings were budded on the 15th of every month

From the above data it may be seen that budding may be carried out almost throughout the year with different degrees of success. The period from November to January seems to be the optimum season and September and February seem to be the next best. Protracted dry and wet weather during the summer months are unfavourable for budding. Under wet conditions fairly good results may be obtained by employing paraffin coated wrappers which prevent the rain water from getting into the slit, but during dry weather no protection can be rendered to a freshly inserted bud which frequently dries and shrivels up. In Madras, July to September is considered to be the optimum season while October to January is considered to be the next best [Naik, 1939]. The position in Assam seems to be almost the reverse. The period of bud-break for the Khasi orange varies from 49 to 62 days and does not seem to show any distinct seasonal variation. Even if there be any seasonable influence it probably gets covered up by the variable character of bud materials as regards the stage of dormancy. It is a matter of experience that the majority of orange buds obtained from the fruiting branch of a seedling orange tree tend to be partially blind and vigilant care has to be exercised in selecting active buds. In a nursery plot for mass propaga-tion when the selection of buds was left to the discretion of a mali it was found that out of 48 successful buds worked on rough lemon rootstocks only 11 burst out within 60 days and the reminder took anything between 100 and 150 days.

EFFECT OF LOPPING THE ROOT-STOCK AT THE TIME OF BUDDING AND LOPPING IT AFTER THE BUDLING HAS ATTAINED A HEIGHT OF 3 IN.

Information as to when the stock plants should be lopped off so as to secure the maximum growth of the budling is indeed important. A trial was, therefore, conducted with two groups of Kata Jamir seedlings (C. limonia Osbeck) identical in age and origin. The mean height and mean diameter of one group of seedlings recorded just before bud-insertion were 64.03 cm. and 11.59 mm. with a SE of 1.09 and 0.24 respectively. The mean height and the mean diameter of the other group of seedlings recorded on the same day were 65.47 cm. and 11.59 mm. with SE of 1.25 and 0.24 respectively showing that the stock materials for both the groups were statistically identical at a 5 per cent level of significance. Plants of both the groups were budded on 6, January 1942, with budwood obtained from a selected Khasi orange (C. chrysocarpa Lushington) tree by the same operator. Immediately after budding the plants of one group were lopped off at a height of 6 in. above the point of union and the plants of the other group were allowed to grow unlopped till the budlings in each case had attained an approximate height of 3 in. The cut at a height of 6 in. was settled with the idea of cutting back the stub to the bud in a progressive manner, instead of in one single cut, as has been advised by Baily [1920], Davis [1917] and Powell [1930]. The extension growth of the individual budlings was recorded on 5, May 1942, i.e. after an interval of four months from the date of budding.

Table XI

Effect of lopping the root-stock at the time of budding on the percentage of success, period of bud-break and extension growth

Treatment	Number of plants budded	Number of success	Percentage of success	Mean period of budbreak in days	S.E.	Mean extension growth in cm.	S.E.
A. Stock lopped at	32	25	78.12	47:24	1.09	36·16	1.14
budding  B. Stock lopped after budling attained 3 in. in height	32	27	84.37	58:30	1.26	47·26	1.57

Note— When these treatments are compared at 5 per cent level of significance the conclusion is as follows:

Period of bud-break— B is significantly greater than A

Mean extension growth—B is significantly greater than A

The above results tend to show that the lopping of the stock plants at the time of budding may lower the percentage of bud-take slightly and induce an earlier bud-break by about 11 days. But notwithstanding the advantage of an earlier

bud-break the amount of extension growth of the budlings, attained in a period of four months from the date of budding, is significantly less in lopped plants than in those where lopping was done after the budlings had attained a height of 3 in. The results are in accord with those of Naik [1939] working in Madras.

Lopping at the time of budding should also be discarded as it causes serious injury to the stock plants by interfering with photosynthetic activities [Mendel, 1937]. Many of these plants develop symptoms of root-rot and wilting and may

ultimately die. This is, however, different from the 'bud-wilt' reported by Halma [1941] as in his case only the budling shows wilting leaving the stock plant unaffected. The degree of tolerance to lopping seems to vary from variety to variety; rough lemons being particularly susceptible and the shaddocks being specially resistant.

Table XII

Extent of mortality caused by lopping at budding on rough lemon and shaddock rootstocks

Scion	Rootstock	Date of budding	Number of plants lopped at budding	Number of dying plants	Percentage of mortality	Remarks
Khasi orange						
(C. chrysocarpa Lush.)	Rough lemon	10. 9.41	17	5	29.41	
_Do	Shaddock	21. 9.41	15	1	$6 \cdot 67$	
—Do.—	Rough lemon	10.10.41	15	4	26.67	
—Do.—	_Do	15.10.41	14	9	$64 \cdot 29$	Irrigated
_Do	—Do.—	24.6.42	33	25	75.60	
Valencia (C. sinensis Osbeck)	Do.—	26. 6.42	15	10	66.67	
Khasi orange	Shaddock	30. 6.42	20	2	10.00	

The data furnished above are not the outcome of any planned experiment but these were collected from the general nursery where lopping at budding was practised with a view to inducing early budbreak. It may be seen that mortality is more during summer months than during autumn. But even during autumn mortality increases quite considerably if the lopped plants are subjected to irrigation. General experiences in the nursery tend to show that all Citrus rootstocks exhibit this intolerance to lopping at budding though some stand it better than others. Since lopping at budding does not exercise any favourable influence either on the percentage of bud-take or on the extension growth of the budling, there seems to be no justification for adopting it as a nursery practice simply because it happens to induce an earlier bud-break. The best course to adopt is to cut back the stock to the bud in a progressive manner allowing some leaves of the stock plant to carry on essential photosynthetic activities till the budling develops enough leaves to take up this essential physiological function exclusively.

#### SUMMARY

1. The extent of seeds producing more than one seedling each in nine citrus varieties has been determined. Such seeds usually produce two seedlings each and sometimes more. Seeds of shaddocks and citrons appear to be monoembryonic.

2. The experiments on the effect of the stage of maturity of fruits of the Khasi orange (C. chrysocarpa Lush.) on the germinating capacity of

seeds show that seeds of mature fruits seem to give a better germination than those of immature ones. Overripening of fruits lowers the viability of seeds to a small extent and prolongs the period of germination quite considerably. Immaturity of seeds has probably a more detrimental effect on the gametic than on the nucellar embryos.

3. Fresh seeds of the Mandarin orange ensure the highest germination which progressively deteriorates with increased doses of sunning.

4. An observation carried out on the viability of seeds with three grades of seed sizes and stored in a desiccator for  $2\frac{1}{2}$  months shows that the bigger the size the longer is the period for which the seeds remain viable and that the viability of seeds progressively deteriorates with the lengthening of storage periods.

5. Investigations into the possibilities of raising plants of citrus from root cuttings planted in open beds show that only citrons and lemons may be propagated by this method with appreciable success. Shaddocks, Seville orange, Mandarin orange and C. macroptera fail to form shoots from planted root cuttings.

6. Treatment with 'Hortomone A' causes a higher percentage of survivals in stem cuttings of those citrus varieties which are ordinarily amenable to propagation from cuttings but it fails to induce rootings in a variety which does not usually root from cuttings. This chemical, however, seems to have some stimulating influence in accelerating both root and shoot formation.

7. All citrus varieties may be successfully propagated by 'gootee'. Citrons, lemons and

C. macroptera put out roots much more quickly than the tight-skinned and loose-skinned oranges,

Seville oranges and grapefruits.

8. The best season for bark-grafting is during July and August, i.e. during rains but the success depends on the continuity of wet weather. The failure of this method during winter months is attributed to the absence of active growing conditions in the stock plants and to the attendent low humidity. Bark-grafting is particularly suitable in inducing an earlier feathering in grapefruit which when budded takes an unusually long period, 3-6 months for bud-break.

9. Of the different methods of shield-budding tested both inverted 'T' and normal 'T' with a minute piece of wood adhering to the bud-shield have been found to give a higher percentage of success than when bud-shields are used with the

wood removed.

The optimum season for budding under conditions obtaining at Burnihat, Assam, is from November to January, i.e. during winter; September and February seem to be the next best.

11. The period of bud-break for the Khasi orange (C. chrysocarpa Lush.) varies from 49 to 62 days and does not seem to show any distinct

seasonal influence.

12. The practice of lopping the stock plants at the time of budding induces an earlier budbreak but lowers the percentage of bud-take and retards the extension growth in comparison with lopping after the budlings have formed few welldeveloped leaves. The former method causes serious injury to the stock plants, many of which develop symptoms of root-rot and consequent. The best course to adopt in a nursery is to cut back the stock plant to the bud in a progressive manner.

#### ACKNOWLEDGEMENT

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## PHOSPHATE STUDIES ON THE PUNJAB SOILS

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(With plate VI and three text-figures)

India exports on an average about 100,000 tons of bones every year, the province of the Punjab alone contributing roughly 23,000 tons. On the basis of 25 per cent  $P_2O_5$  available in bones, the Punjab soils are deprived every year of about 6,000 tons of this important chemical, and thus the export of bones means a loss of great potential wealth to the Punjab agriculturist.

Nitrogen and other plant food-constituents are added to the soil in a number of ways; recuperation of nitrogen may even be effected by natural processes but there exists no such agency for returning phosphates to the soil. It is not surprizing, therefore, that attempts should have been made from time to time to utilize bone manures, this being the only indigenous phosphatic manure of the province, for improving the phos-

phate status of the Punjab soils. The Department of Agriculture, Punjab, has been carrying out trials with artificial fertilizers, but in the majority of earlier experiments, phosphates either alone or in conjunction with other manures did not improve the yield of the crops to an extent which would justify the use of these manures on an economic basis. Later on, experiments were carried out with different grades of bone meal on wheat and cotton. It was observed that the addition of bone meal either singly or in combination with other materials did not increase the yield nor was there any residual effect on the succeeding crops. As a result of these experiments it was concluded that Punjab soils were quite rich in phosphates, and they did not require any treatment with phosphatic manures. The more recent work on the Departmental farms, however, indicates that soils from the sub-montane tracts such as Rawalpindi and Gurdaspur do show a response to phosphatic manures. Therefore, an investigation was taken up to study the phosphate status of the soils of the Punjab, viz. their power of phosphate fixation, movement of phosphate through calcareous soil, and the response of calcareous and sub-montane types of soil to phosphate fertilization at different depths. results of the investigation have been reported in

#### REVIEW OF EARLIER WORK

this paper.

McGeorge and Breazeale [1932] report that the native soil phosphates in calcareous soils are of the carbonato-apatite group [3Ca<sub>3</sub> (PO<sub>4</sub>)<sub>2</sub> CaCO<sub>3</sub>], whose solubility is a function of hydrogen ion

concentration, the least being at a pH of 8.0 to 8.5. Hibbard [1935] described a number of factors affecting the fixation of phosphate, and considers calcium carbonate as a powerful agent of fixation. Midgley [1931] has found that superphosphate applied to the surface soil moves very slowly with irrigation water. Hockensmith [1923] has found a great response to phosphate application at a depth which is favourable for the absorption of phosphates by roots.

#### METHODS OF ANALYSIS

Phosphorus. This was estimated in extracts of suitable concentration by the ceruleo-molybdate method of Denigé as modified by Truog and Meyer, using the photoelectric colorimeter for colour measurements.

Available phosphorus. This was determined in Way's 1 per cent citric acid and Truog's 0.002N H<sub>2</sub>SO<sub>4</sub> extract (buffered with ammonium sulphate).

Plant analysis. This was carried out according to the Methods of analysis of the Association of Official Agricultural Chemists.

#### PHOSPHATE STATUS OF PUNJAB SOILS

In view of the continued removal of phosphates by farm crops from the soils without any corresponding addition in the form of manures, it becomes a matter of great concern to know the comparative content of phosphates in the different types of soils of the province. Total phosphorus and some of the important pedological characters of six typical Punjab soils, representing different climatic groups is presented in Table I.

Table I

Analysis of some typical Punjab soils

Place	Soil type	Total phosphorus* (parts per million)	Available phosphorus; (parts) per million)	CaCO <sub>3</sub> (percentage)	Clay (percentage)	pH;
Palampur (Kangra)	Hill soil	340	25.6	nil	25 · 27	5:0
	Sub-montane	549	32.8	nil	26.30	6.8
Lyallpur	Alluvial	1207	366	2.85	15.83	8.0
	Calcareous	811	146	12.35	49.08	7.8
Montgomery	'Bari'.	1434	329	4.40	21.70	9.66
Lyallpur	Saline	832	143	5.56	15.23	9.18

\*Total phosphorus estimated by the acid hydrofluoric method

†Available phosphorus estimated by extraction with 1 per cent citric acid. ‡pH determined with Beckmann's Glass electrode pH-meter It will be seen from the above data, that the quantity of total phosphorus varies within wide limits. The Palampur and Rawalpindi soils contain comparatively much less total and available phosphorus than others. This deficiency is also indicated by the results of field experiments conducted at Rawalpindi, where the application of superphosphate at the rate of 25 lb. P<sub>2</sub>O<sub>5</sub> per acre gave an increase of 5 md. of wheat per acre. In order to find out whether other mountain and sub-montane soils are also deficient in available phosphorus, soil samples from the mountainous tracts were also analysed for their available phosphorus

The results of these analyses are presented in Table IIA and B.

## TABLE II A

Available phosphorus content of some more Punjab soils
Group A: Mountain and sub-montane regions

	Local	ity	
Palampur fruit garden	Palampur potato farm	Palampur potato farm	Gurdaspur
50*	87*	87*	64*

# \*Available phosphorus (parts per million)

TABLE II B

Available phosphorus content of some more Punjab soils
Group B: Central plains

	Localit	t <b>y</b>	
Sheikhupura	Lahore	Ferozepur	Hissar
236*	302*	216*	195*

\*Available phosphorus (parts per million)

The above figures indicate that the soils of mountain and sub-montane groups are also highly deficient in available phosphorus. It may be noted that the phosphorus in the soil exists in different forms; in acid soils (pH 5.0-6.8) phosphorus exists in the form of AlPO4 and FePO4, while in the neutral and alkaline soils (pH7.0-8.5) it is present in the form of calcium phosphate Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>. In the former types of soils not only is the total phosphorus content less but the iron and aluminum phosphates being less soluble, the replenishment of the phosphate in the soil solution is comparatively much less which leads to a great phosphorus deficiency. Shedd [1921] has given the limits (0.08 to 0.10 per cent total phosphorus) where phosphate applications will be useful, and according to him the soils of the mountain and submontane tracts of the Punjab fall in the category of deficient soils.

DISTRIBUTION OF PHOSPHORUS IN THE SOIL PROFILE

Dunnewald [1929] has shown that in cultivated soils the amount of available phosphorus is at its maximum in the surface layer and that this figure gradually decreases, with increasing depth of the soil. It may be noted that his finding offers a striking contrast with the distribution of potassium in the soil profiles where no such variations are observed. In order to see how the phosphates are distributed in the cultivated soils of the Punjab, samples representing different depths of profiles were obtained from some typical soils and the amounts of total phosphorus were determined in each case. The results are shown in Table III, A and B.

Table III A

Hel-soluble phosphate content of two profiles

			Percentage	in air dry soil			
Soil	1st 6 in.	2nd 6 in.	2 ft.	3 ft.	4 ft.	5 ft.	6 ft.
Montgomery normal $\frac{P_2O_5}{K_2O}$	0·164 0·730	0·159 1·070	0·150 0·570	0·141 0·630	0·138 0·590	$0.143 \\ 0.710$	$0.153 \\ 0.725$
Montgomery 'bari', $\begin{array}{c} P_2O_5 \\ K_0O \end{array}$	0·197 0·690	0·185 0·925	0·167 0·755	0·161 0·855	0.141	0·134 0·980	0·123 1·000

TABLE III B

Available phosphorus content of some profiles

	Phosphorus parts per million						
	1 ft.	2 ft.	3 ft.	4 ft.	5 ft.	6 ft.	
Cultivated Ferozepur Gurdaspur Montgomery	255 64 189	138 29 138	138 29 80	22 29 36	Trace 22 29	Trace 14 29	
Uncultivated Ferozepur Montgomery Lyallpur	60 110 63	60 108 82	30 95 71	25 82 62	15 30 25	Trace 18 12	

The results given in the above tables are in full agreement with those reported by Dunnewald [1929]. In the case of both the soil profiles, viz. normal and 'bari', the amount of total as well as available phosphorus decreases with increasing depth of soil, the change being more pronounced in respect of available phosphorus. The distribution of potash behaves in a different manner. It is not possible to offer any detailed comments on the manner of distribution of these two important elements of soil fertility but obviously the accumulation of a high percentage of phosphorus in the upper layer cannot be due to an upward movement of phosphates along with other water soluble salts but it may be ascribed to the action of growing plants which collect the phosphates from the lower layers and, through the plant residues after decomposition, they are left behind on the surface where they may remain bound up with the soil due to its fixing power. In the case of potash salts, owing to leaching, the amounts present in the lower layer are more. In order to throw further light on the above assumption a number of samples of cultivated and uncultivated soils were obtained from different localities in the Punjab and the amount of available phosphorus determined in them. The results of these analyses are given in Table IV.

Table IV

Available phosphorus content of adjacent cultivated and uncultivated (barren) soils

	Ph	osphorus	(parts per	million)	
Soils	Sheikhu- pura	Lahore	Feroze- pur	Feroze- pur	Hissar
Cultivated Uncultivated	236 101	302 146	106 53	221 36	195 123

In all cases the available phosphorus is higher in the cultivated area, inspite of the large amounts removed by the crops each year. This must be due partly to the above mentioned action of the cultivated plant, and partly due to other factors such as manuring and cultivation.

#### FIXATION CAPACITY OF PUNJAB SOILS

Since fixation capacity of soil is an important factor in the application of phosphatic fertilizers, it was deemed necessary to have some idea of the extent and nature of this factor in the case of the Punjab soils, since the ultimate use of these fertilizers must depend on their availability after being added to the soil, and the extent to which they are in a position to move with the soil solution.

Fixation capacity of some of the typical soils were measured by the method of Heck [1934], and are reported in Table V.

Table V
Fixation capacity of some Punjab soils

Place	Phosphorus added (parts per million)	Phosphorus in filtrate (parts per million)	Percentag fixed
Palampur	400	146	63 · 5
Rawalpindi	400	320	20.0
Lyallpur	400	348	13.0
Dhundi	400	278	30.5
Bari	400	362	9.5
Saline	400	312	22.0
Sargodha	400	309	22.7
Sheikhupura	400	336	16.0
Lahore	400	371	7 · 2
Lahore	400	353	11.7

Majority of the soils allowed 309 to 371 parts of phosphorus out of 400 parts per million to pass into the filtrate, only Palampur and Dhundi Estate soils allowed 146 and 278 parts respectively. Palampur soil has developed under humid climate (rainfall about 90 in.) with excessive leaching. It contains large amounts of free aluminum and ferric oxide which are very reactive in fixing phosphates from solution. Dhundi soil, on the other hand, is highly calcareous, and possesses a high fixing capacity due to the presence of large amounts of calcium carbonate.

The method of measuring fixation is an arbitrary one, as the amount of phosphate fixed depends on a number of factors. The effect of two important factors namely the concentration of PO<sub>4</sub> in the solution, and the presence of CaCO<sub>3</sub> in the soil were further investigated. For this study 7·5 gm. of each of the three soils (Palampur, Rawalpindi and Lyallpur) were taken and increasing quantities of phosphorus were added in the form of H<sub>3</sub>PO<sub>4</sub> to each, keeping the volume the same in all cases (50 c.c.) The suspensions were shaken occasionally for 24 hours, after which they were filtered and phosphorus determined in the filtrate. The amounts of phosphorus fixed for each concentration are graphically represented in the Fig. 1.

The actual amount fixed as also the percentage of the total added are shown in Table VI.

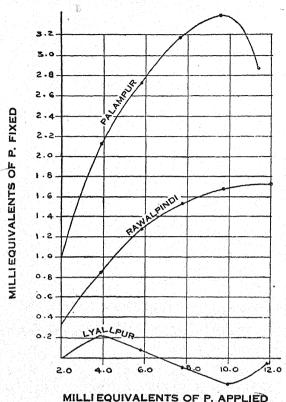


Fig. 1. Phosphorus fixation in different Punjab soils

Table VI

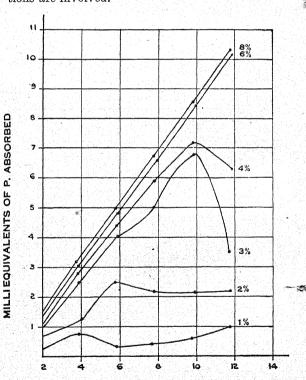
Percentage fixation of phosphorus with increasing concentration of phosphate solution

		<b>P</b> !	hosphorus	s fixed				
Phosphorus added (milliequi- valents)	Palan	Palampur Rawalpindi				Lyallpur		
	Milli- equivalents	Percentage	Milli- equivalents	Percentage	Milli- equivalents	Percentage		
1.946	1.153	59.3	0.334	17.1	0.012	6.20		
3.892	2.092	53.7	0.843	21.7	0.246	6.32		
5.838	2.687	46.6	1.272	21.8	0.066	1 1 13		
7.784	3.104	39.9	1.492	19.2	-0.057	-0.73		
9.730	3.360	38.5	1.644	16.8	-0.202	-2.08		
11.676	2.885	24.6	1.653	14.2	0.024	0.21		

These figures also show that Palampur soil possesses the highest phosphorus fixation capacity, and the amount of phosphorus actually fixed in

these cases goes on increasing up to a certain limit beyond which it begins to fall. In the case of Lyallpur soil when the amount of phosphorus added is more the fixation becomes even negative. It may be remarked that these results agree with those obtained by Ravikovitsch [1934]. The percentage of phosphorus fixed however varies indirectly, i.e. with increasing concentrations, the percentage of phosphorus fixed goes on decreasing.

Since most of the arid and semi-arid soils in the Punjab contain varying amounts of CaCO3, the effect of this substance on their fixation power was also studied. A series of soils containing varying amounts of CaCO3 was prepared by mixing requisite quantities of calcium carbonate. soils were then wetted to optimum moisture content and left for 48 hours. They were then dried, pulverized and used for the estimation of the fixation power as before, adding increasing quantities of phosphorus in the form of a standard solution of H<sub>3</sub>PO<sub>4</sub>. The data are graphically presented in Fig. 2. It shows that the fixation power of the soil is largely affected by calcium carbonate and bears relationship with the amount of phosphorus added, it being less at lower concentrations but pronounced when higher concentrations are involved.



MILLI EQUIVALENTS OF P. APPLIED

Fig. 2. Effect of calcium carbonate on phosphorus fixation

#### PHOSPHATE PENETRATION

Since soil acts as a powerful adsorbing agent for  $PO_4$  ions, and further under field conditions the dose of phosphatic fertilizers is comparatively low as compared with that used in the experiment described above, it would be expected that large proportion of the added phosphate would be absorbed, and made immobile. Under these circumstances movement of phosphate would be restricted. In order to study the penetration of phosphate when applied as a top dressing, the following experiment was arranged.

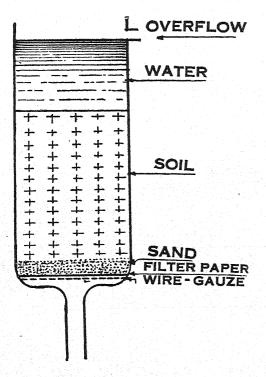


Fig. 3. Diagram of percolation tube

A series of brass percolation tubes was fitted with wire gauze, filter paper and a layer of sand at the bottom, as shown in Fig. 3. Into these tubes, soil in varying amounts was uniformly packed in order to give columns of different heights. Superphosphate, 0.02 gm., was sprinkled on the surface as top dressing. Distilled water corresponding to 6 in. irrigation was added on the top and allowed to percolate, the water filtering through was collected in various fractions and P<sub>2</sub>O<sub>5</sub> content estimated by the colorimetric method. The results are shown in Table VII.

TABLE VII

Penetration of superphosphate through Lyallpur soil

Weight of soil used (Approximate height of column)	100 gm. 3 in.	200 gm. 6 in.	300 gm. 9 in.	100 gm. sand 3 in.
Fractions 1st 100 cc. 2nd 100 cc. 3rd 100 cc.	14* 3·5* 3·0*	5* 3* 3*	3* 3* 3*	60* 60* 60*
Calculated percentage of total superphosphate moved with 6 in, irrigation	3.75	0.75	0.22	28.0

\*Concentrations of phosphorus in leachings (colorimetric readings)

From the above data it appears that the percentage of super phosphate moving through different layers of soil with a 6 in. irrigation is very small, decreasing considerably as the column of soil increases in thickness. The first 100 c.c. fraction in the case of 3 in. layer had a much higher concentration than others, but in later fractions the concentration was the same in all cases thus indicating that the higher concentration in the first fraction of the 3 in. layer is mainly due to initial infiltration of fertilizer particles which ceases afterwards. That this resistance to penetration through soil is due to the colloidal nature is proved by comparing the above results with those obtained in the case of sand. These data lead to the obvious conclusion that since phosphatic manures do not move much with irrigation water, they should best be applied at a depth most suited for absorption by the roots.

#### Pot experiments

# Depth of application and crop response

Phosphatic fertilizers are known to influence plants in the better development of the root system, better grain formation, earlier maturity resulting in increased yields. The effects produced are, however, proportionate to the phosphate deficiency in the soil. Field experiment with the application of phosphatic manures to the Punjab soils have however shown that generally speaking the soils of the Punjab plains do not respond to an application of phosphatic manures. The only cases in which certain response was observed, related to soils of the sub-montane tracts of Rawalpindi and Gurdaspur. As already mentioned, this latter group of soils also appears to be deficient according to the laboratory tests; the former group however does not fall into this category. It may be pointed out that in the field experiments

fertilizer was used as a top dressing in small doses. Kellog [1931] also states that calcareous soils such as are found in arid and semi-arid regions, and apparently possess a high content of phosphorus do not respond to low doses. Norris [1922], working on Madras soils also observed that increased yields were obtained only after continuous manuring with phosphates. These findings point to the necessity of supplying higher doses of phosphates to the Punjab soils. Besides, it has already been remarked that phosphates applied to the soil as top dressing do not penetrate into the subsoil to any appreciable extent. Therefore, a series of pot experiments on two types of soil using two different crops, was designed to test the availability of phosphates when applied in high doses, and to find out the most suitable depths at which these manures would be most effective.

Scheme of pot experiments

In the following series of experiments a uniform procedure was followed. 3,000 gm. of air dried, and sieved soil was packed in cylindrical glass pots 13 in. high and provided with a tubular at the base for aeration. The fertilizer was applied to these pots at varying depths in known quantities. Throughout the experiment the moisture percentage in each pot was maintained at a uniform

level, the initial watering being done on the basis of the moisture equivalent of the soil. Tomato or wheat seedlings were transplanted into these pots. A record of growth measurements such as height, number of tillers, date of fruiting, etc. was kept for each pot. At the stage of maturity the plants were removed from the base, their green weights and oven dry weights being recorded.

Ravalpindi soil. The experiment was started on 21-10-43, using tomato as a test crop which according to Fisher [1935] is very sensitive to phosphatic manures. Single superphosphate at the rate of 400 lb. per acre, was applied and the following treatments were included, each being

in three replicates:

(1) Control

(2) Application at the surface
(3) Application at 3 in. depth
(4) Application at 5 in. depth
(5) Application at 7 in. depth

Only two plants were allowed to grow in each pot, and on the ripening of the fruits they were harvested on 27-I-44. A photograph was also taken depicting their comparative growth at maturity (Plate VI, fig. 1). The green weight of each plant was determined, the weight of the fruit was also ascertained separately. The summary of the data is presented in Table VIII.

Table VIII

Growth, yield and analysis of tomato crop from pot experiments with Rawalpindi soil

	Height of plants in cm.		Green wt.	Total wt.	Wt. of fruit	Oven-dry wt. of treated	Percentage	Total P2O
Treatments	Plant No. 1	Plant No. 2	of plants in gm.	of treated plants in gm.	in gm.	plants in gm.	P <sub>2</sub> O <sub>5</sub> in oven-dry material	removed in mg.
Control	23·0 24·0 16·0	16·5 18·5 30·5	8·0 10·8 20·2	39.0	nil	4.963	0.554	27.5
Surface application	41·5 28·0 32·0	40·5 41·5 33·5	29·1 29·4 25·7	84•2	6.2	11 · 995	0·490	58.8
3-in. depth	30·5 32·0 36·5	36·0 42·0 41·0	50·2 24·0 42·0	116-2	19·7	17:991	0.543	97:6
5-in. depth	47·0 41·0 38·0	45·0 39·0 43·0	50·0 37·0 43·0	150.0	5:0	19·812	0.458	90.7
7-in, depth	30·0 21·0 24·0	33·0 29·0 31·0	29·8 33·0 31·4	94+2	nil	12•753	0·464	59·2

It was observed that in the case of control pots, the tomato plants almost invariably showed a peculiar purple coloration on the lower side of the leaves. Cunningham [1922] made a similar

observation that phosphate-starved barley plants grown in water cultures develop a reddish colour on the stems and a sickly purple in the leaves The yield data shows that though surface application gives an increase in the yield of dry matter over the control, yet this increase is much less than the increase obtained with applications at 3 in. and 5 in. depths. Besides, the fruit formation is also the best in the case of application at 3 in. depth. The figures show that the unmanured plants contain the highest percentage of  $P_2O_5$ , yet the total quantity of  $P_2O_5$  removed by the plants was higher in the case of 3 in. and 5 in. depths.

Lyallpur soil. The experiment was conducted on the same lines as that with the Rawalpindi

soil. The application of manures was made at the surface, at  $2\frac{1}{2}$  in.,  $4\frac{1}{2}$  in. and  $6\frac{1}{2}$  in. depths. Instead of tomato the experiments were conducted with wheat. The method followed was the same as in the previous experiment. Two plants were kept as in the previous experiment. Seedlings were planted on 23-12-43, and the mature crop was harvested on 24-4-44. After the fresh and oven dry weights of the plant material had been recorded, the plant material was ashed and analysed for insoluble residue, calcium and phosphorus. The growth data and the results of analysis are given in Tables IX and X.

Table IX
Growth and yield of wheat crop from pot experiments with Lyallpur soil

Treatment	Average height per tiller in cm.	No. of tillers	Green wt. per treatment in gm.	Dry wt. per	Grain/straw	
Treatment	per uner in cm.	1,0. Of Miles	oreasment in gin.	Grain	Straw	ratio
Control Surface $2\frac{1}{2}$ in. depth $4\frac{1}{2}$ in. depth $6\frac{1}{2}$ in. depth	56·5 61·5 61·9 60·5 58·5	16 16 18 17 16	39·5 41·1 46·8 43·2 41·5	12·85 13·37 16·60 14·77 14·30	15·70 16·40 17·25 16·60 16·40	0·819 0·815 0·962 0·890 0·872

Table X
Proximate analysis of grain and straw of wheat from the pot experiments with Lyallpur soil

		I	Percentage on o	Comparative yields			
Treatment		Ash	Insoluble residue	P, O,	P,O, CaO	Grain	Straw
Control	Grain Straw	1·64 9·06	0·30 4·68	0·654 0·047	0·113 0·489	100	100
Surface	Grain Straw	1·73 8·65	0·15 4·55	0.800 0.062	0·084 0·492	104	104
$2\frac{1}{2}$ in. depth	Grain Straw	1·78 9·37	0·18 4·92	0·944 0·081	0·115 0·688	129	110
$4\frac{1}{2}$ in. depth	Grain Straw	1·71 8·35	0·16 4·24	0·924 0·078	0·121 0·516	115	106
$6\frac{1}{2}$ in. depth	Grain Straw	1·82 8·44	0·17 4·62	0·858 0·066	0:110 0:492	111	105

It is clear from the data that application of superphosphate has produced an increase of yield in all cases, and has also increased the phosphate content of the plants. As already observed the surface application has also responded, but the response is much less than that obtained with deeper applications. The main points are summarized below:

1. Average height of tillers has been increased over controls in all cases of phosphate application,  $2\frac{1}{2}$  in depth producing the highest tillers. In other

cases the height of tillers decreased with the increasing depth of application. This can be judged from the photograph of the pots (Plate VI, fig. 2).

fig. 2).

2. Application at a depth of  $2\frac{1}{2}$  in. gave a 29 per cent increase of grain over the control, but only a ten per cent increase in the case of straw. Grain to straw ratio becomes much higher in the case of  $2\frac{1}{2}$  in depth than in other cases.

the case of  $2\frac{1}{2}$  in. depth than in other cases.

3. Application of superphosphate has greatly increased the percentage of  $P_2O_5$  in the grain,

although there is not much difference in the percentage of CaO.

### DISCUSSION OF RESULTS

Both the experiments have revealed the fact that great increase in yields can be obtained by applying phosphate at a suitable depth instead of applying it as a top-dressing, a practice usually in These studies have also shown that phosphate applied at the surface is fixed up in the soil and is unable to move into deeper layers with irrigation water. The effective root zone of the majority of field crops lies between 3 in. to 12 in. layer, and it is most essential that the manure applied should be available in the root zone. The most convenient depth which has given the best results under the conditions of the experiment is 3 in. to 5 in. This experiment is being extended in field conditions, in which case the manure is being applied at various depths by dropping it in the furrow.

#### SUMMARY

1. According to the existing methods of estimating available phosphorus, the Punjab soils may be classified into two main-groups, (a) the mountain and the sub-montane soils which are deficient in available phosphorus, and (b) the arid and semi-arid plains which appear to be fairly well supplied with phosphorus.

2. The distribution of total and available

phosphorus shows that the surface layer contains the maximum amount of phosphorus, both total and available, and that these amounts decrease with increasing depths.

3. The fixation capacity of different soils varies with the nature of soil and the amount of CaCO3

present.

4. Pot experiments both with calcareous and non-calcareous soils have shown that application of phosphate at a depth of  $2\frac{1}{2}$  in. to  $4\frac{1}{2}$  in. gives a better response than the surface application.

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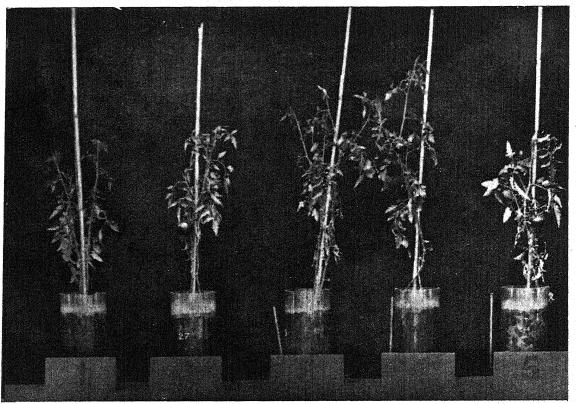


Fig. 1. Effect of phosphate placement in Rawalpindi soil. Crop: Tomato.
1. Control; 2. Surface application; 3. Application at 3 in.; 4. Application at 5 in.;
5. Application at 7 in.

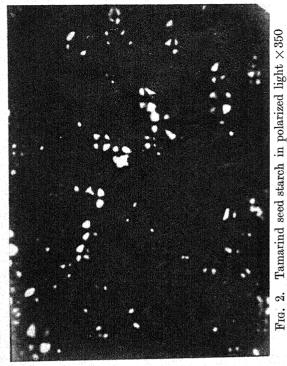


Fig. 2. Effect of phosphate placement in Lyallpur soil. Crop: Wheat.

1. Control; 2. Surface application; 3. Application at  $2\frac{1}{2}$  in.; 4. Application at  $4\frac{1}{2}$  in.;

5. Application at  $6\frac{1}{2}$  in.

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Tamarind seed starch in polarized light  $\times 350$ 

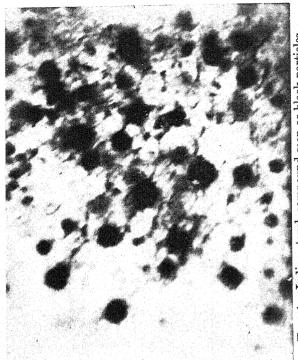
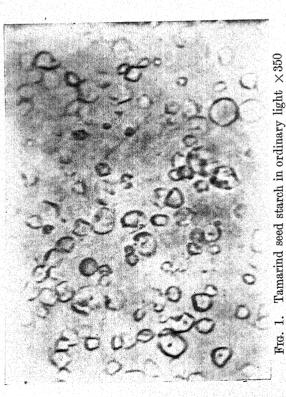
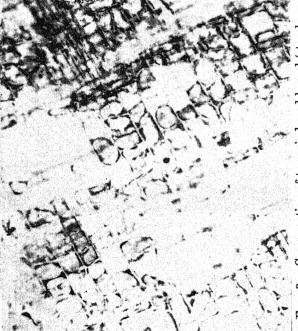


Fig. 4. Iodine-starch compound seen as black particles in the cells  $\times 350$ 





Cross section of tamarind seed kernel (starch granules embedded in cells)  $\times 350$ Fig. 3.

# CHEMICAL EXAMINATION OF THE SEEDS OF TAMARINDUS INDICA NO PECTIN IN TAMARIND SEEDS

By Raj Narain and Sikhibhushan Dutt, Chemical Laboratory, University of Delhi (Received for publication on 28 March 1945) (With Plate VII)

TAMARINDUS indica or the Tamarind tree is a large deciduous tree that is found throughout India both in the wild and the cultivated state. It is a handsome ever-green tree with large spreading branches and slender leaves, often growing to a height of 70 to 80 ft. It is often cultivated as a road side tree for its cool shade, but the well known fruit pods of the plant, with their delicate acid flavour, are its most commercially valuable

products.

According to Dymock [1893], the fruit is an oblong or linear oblong, slightly compressed, curved or nearly straight pendulous legume, of the thickness of the finger, 3 to 6 in. in length, supported by a woody stock. It has a thin but hard and brittle outer shell or epicarp which does not split into valves or exhibit any evident sutures. Within the epicarp is a firm, acid juicy pulp, on the surface of which, and starting from the stalk, are ramifying nerves; one of these extends along the dorsel or concave edge, two others on either side of the ventral or convex edge, while between these two there are usually 2 to 3 or 4 less regular and more slender nerves all running towards the apex and throwing out branching filaments. The seeds are 4 to 12 in number and are enclosed in a tough membraneous covering, surrounded by the pulp.

The Indian commercial article forms a firm black sticky mass. With the pulp are mixed the seeds, fibres and a small fragment of the shell. It is usually salted but for pharmaceutical purposes

it should be free from salt.

The preserved pulp of the tamarind fruit is officinal in the British Pharmacopoea. It consists of a reddish brown sugary mass enclosing the stringy fibres founded within the pulp and the seeds enclosed in a tough membraneous coat. A corresponding product often occurs in India as a black solid mass of the pulp, more or less free from the fibres and husk, pressed into round cakes and preserved with salt.

Tamarind pulp contains large quantity of sugar and organic acids like acetic, citric and praticularly tartaric acid which latter it contains often to the extent of 6 to 8 per cent. The tartaric acid present is partly free and partly in combination with potassium in the form of potassium hydrogen tartarate or cream of tartar. No particular principle to which the laxative action of tamarind

is due is known.

The seeds of the tamarind fruit are small dark

brown glossy nuts often rectangular in shape but rarely oval or round. They are about 12-15 mm. in length, 8-12 mm. wide and 3-5 mm. in thickness. The outer facets are slightly convex. The pericarp consists of a hard shell or covering enclosing a hard white kernel. The seeds are said to have good astringent properties. They are applied as poultice to boils after being boiled. The seeds are also crushed and pounded with water and applied to the crown of the head in cough and relaxation of the uvula.

The seeds, boiled or fried after removal of the outer skin, are also eaten by poor people in Malabar, Madras, Andhra, Bengal and Central Provinces particularly in times of a scarcity. Cameron [1894] mentions a cement or paste as made from the seeds which is used in dressing country-made blankets. In Central Provinces and Madras the seeds after frying and removal of the outer husk are ground to a flour in hand driven stone mills, and the products used in the same way as flour or ata from wheat or maize. It is stated that a number of sweetmeats are also made out of the preparation. According to Yunani medicine, the seeds are astringent, aphrodisiac, useful in giddiness and vertigo and applied externally in liver complaints and inflammation. They are also used in diarrhoea.

In view of the medicinal importance of the seeds it is very desirable that their chemical composition should be known with certainty. The first chemical examination of the seeds was done by Ghosh and Krishna [1942], who found that tamarind seeds consists of 55 per cent of kernels and 45 per cent of testa. The kernels on being analysed

were found to contain:

Moisture	10.20 gm. per cent
Albuminoids	15.40 ,
Oil	6.4
Crude fibre	5.0 ,
Sugars	2.9
Tannins	1.6
Pectins	58.5
Ash	2.5 $,,$
Total	102.5

In the same paper, Ghosh and Krishna made the most sensational announcement that the tamarind seed is a very rich source of commercial pectin, containing as it does nearly 64 per cent of this material in the dry weight of the kernel which can be easily extracted on a commercial scale by following simple methods. They also showed that the pectin isolated in this way was quite pure, as it formed a stiff jelly when 1.5 gm. of the material was boiled with 65 gm. of sugar and 1 gm. of citric acid in 100 c.c. of water. The jelly did not break or run even when kept standing for over a month under proper sterile conditions.

In reviewing the work of the above authors, regarding the percentage of pectin in tamarind seed the present authors were surprized to note that whereas according to the analysis of Ghosh and Krishna, the percentage of pectin in the seeds was as high as 58.5, that of moisture was only 10.2. This is most extraordinary and yet unknown in the vegetable kingdom. For pectin is a substance like gelatin or agar-agar which is capable of retaining a large amount of moisture, and in nature pectin is always associated in fruits and vegetables with a large amount of moisture which is generally never less than 70 per cent of the total weight of the fruit or vegetable as shown in Table I.

Table I
Water and pectin contents of fruits or vegetables

Fruit	or vegetable	Percentage of moisture (gm.)	Percentage of pectin as calcium pectate (gm.)
Apple	(Pyrus malus)	87	1.5-2.5
	(Citrus medica)	85	$2 \cdot 5 - 4 \cdot 0$
Lemon peel		77	3 · 6 – 3 · 8
	o (Citrus aurantia)	85	3.5-5.5
Orange pee		76	3.5 - 4.2
Beet-root	(Beta vulgaris)	92	1.0
Carrot	(Daucus carota)	93	0.62
Onion	(Allium cepa)	92	1.1-2.2
Leaves	(various kinds)	70-87	0.6 - 1.2

Singh and Dutt [1941] working on the formation of jellies from Indian fruits have also shown that pectin yielding Indian fruits also contain a large amount of moisture as shown in Table II.

TABLE II

Moisture and pectin contents of some Indian fruits

Fruit	of Moisture	Percentage of pectin as calcium pectate (gm.)
Wood apple (Fronia elephantum)	71.8	3.95
Guava (Psidium quava)	78.8	1.44
Karaunda (Carissa carandas) Roselle— (Hibiscus Sabdariffa)	89-0	1.23
Calvx	88.2	3.19
Entire fruit	82.66	2.48
Fruit only	76-4	1.02
Orange (Cirus nobilis)	88.6	1.2
Lemon (Citrus limonum)	85.08	2.76
Jujube (ber) (Zizyphus jujuba)	80.01	1.45
Banana (Musa paradisca)	73.6	1.19
Bel (Aegle marmelos) Cape	68.8	2:03
gooseberry (Physalis peruviana)	83.8	0.75

Further survey of the pectin yielding materials from the vegetable kingdom indicated that pectins are always found in soft tissues of the plant like fruits and stems and pulpy roots and tubers and has never been known to occur in hard tissues like wood or seeds. This is nothing to be wondered at in view of the great water retaining capacity of vegetable pectins and their essentially slimy or pectinous' nature. Hence Ghosh and Krishna's discovery of 58.8 per cent of pectin in the fresh seeds of tamarind must come as a surprize to people acquainted with the occurrence and nature of pectins and pectic substances in general. In fact the discovery was so revolutionary in character and scope, that the present authors were prompted to re-examine the problem and subject the work of Ghosh and Krishna to very careful scrutiny.

On going through the analytical figures of Ghosh and Krishna regarding the fresh seed of tamarind, the present authors were surprized to find that the figures which carry the constituents up to 102.5 per cent, did not have any reference to starch at all in the material. A seed without starch is like an animal without protein, definitely unthinkable. Starch is absolutely essential for the growth of the germ cell and the young plant in the initial stages of its life and there is no seed known which is free from starch. Hence Ghosh and Krishna's analysis of tamarind seed as containing no starch must be regarded as positively erroneous. Even qualitative examination of the tamarind seeds, as we have found by actual experiment by extracting the powdered seeds with hot water and adding iodine solution to the cooled extract, gives a copious indication of the presence of starch by the intense blue colour that is produced. The intensity of the blue colouration that is thus produced with iodine is so great that it is quite reasonable to assume the presence of large quantities of starch in tamarind seeds, and this is not inconsistent with the agelong custom that has been prevailing in various parts of India of using these seeds as food materials. In fact from reports that the present authors have been able to get regarding the value of the seeds as food material, it seems quite apparent that they are used in the same way as starchy cereals like rice, wheat or maize, i.e. staple foods with similar nourishing qualities.

From what has been stated above it seems quite clear that tamarind seeds must contain large quantities of starch which Ghosh and Krishna have entirely missed in their analysis, and that it is quite unlikely that they should contain such a high percentage of pectin as 58.5. Besides the method of estimation of pectin as adopted by Ghosh and Krishna, namely alcohol precipitation method, has been shown to be definitely erroneous by a number of authors like Hinton

[1940], Allen [1937] and others who have pointed out that alcohol precipitates not only pectins but many other substances like albuminoids, starches, organic acids, gums and mucilages, thus making this process of extraction entirely worthless, particularly where pectins are associated with such materials.\* According to most well known authors the quantitative estimation of pectin can be best carried on by the method of Emmett and Carre [1926] as modified by Nanji and Norman [1928] in which the pectic substances present in any plant material are converted into calcium pectate and estimated as such. By following this method the present authors tried to estimate the amount of pectin present in tamarind seeds, but to their great surprize they found that they contained no pectin at all, as no precipitate of calcium pectate could be obtained under any circumstances.

According to Meyers and Baker [1934] pectin in the unhydrolysed condition is mono-arabino-mono-galacto-diacetylhepta-methoxyl-octagalacturonic acid. The nucleous of the pectin molecule is octa-galacturonic acid most likely formed by the union of two molecules of tetra-galacturonic acid with the elimination of one molecule of water. The tetra acid is most likely formed into a ring compound by the combination of four molecules of galacturonic acid with the elimination of four molecules of water. Seven of the eight carboxyl groups of the octa-galacturonic acid are methylated, and the other one is free. On this basis, the empirical formula for pectin would be C<sub>70</sub> H<sub>98</sub> O<sub>58</sub> with a molecular weight of 1,866,784.

Further, Baker and Goodwin [1939] while reviewing the work of various authors have stated that 'Present opinion definitely favours the assumption that pectin is a chain compound composed of galacturonic acid groups. In this chain compound the carboxyl groups are 75 per cent methylated (11.92 per cent CH<sub>3</sub>O) and the position of the free carboxyl is arbitrary'. The constitution of pectins has been fully dealt with by Hinton [1940]. All these show that pectin should contain methoxy groups which can be easily estimated by Zeissel's method. A determination of methoxy groups by the Zeissel's method also gave negative results, showing the complete

\*According to Allen's Commercial Analysis and Hinton's Fruit Pectins, the estimation of pectic substances as calcium pectate, a method first introduced by Carre and Haynes [1922] forms the only satisfactory process for the accurate determination of these substances. Calcium pectate is insoluble even in extreme dilution and is a substance of definite composition into which all pectins are readily converted. Its insolubility in dilute acetic acid allows the removal of the calcium salts of all organic acids commonly met with in plant products except oxalic acid [Allen, 1937]. A detailed method for estimating pectin as calcium pectate has been mentioned by Hinton [1940].

absence of pectin in tamarind seeds. It seems therefore quite apparent that Ghosh and Krishna must have mistaken the larger amount of starch present in tamarind seeds to be pectin. It is a pity that they hastily announced their erroneous discovery to the public without taking trouble to make sure of their grounds by accurate experimentation.

The jellies which Ghosh and Krishna obtained with tamarind seed are undoubtedly starch jels. It is a well known fact that many varieties of starch have extraordinary power of jellification, specially in presence of sugars and several varieties of sweetmeats are commonly prepared in this way by halwaies all over the country. Properly made jellies prepared from pectin are very durable and remain firm for years under ordinary conditions of storage whereas starch jellies last only for a few days unless they are kept under aseptic conditions. It is significant that Ghosh and Krishna have mentioned that the jellies prepared by them from tamarind seeds remain firm for a month only under proper sterile conditions.

Still another error which Ghosh and Krishna apparently made was in the estimation of the proportion of testa and kernel in the seeds. They have given the figures of 45 per cent of testa and 55 per cent of kernel whereas we actually found that the proportions were 30 per cent and 70 per cent respectively.

#### EXPERIMENTAL

Tamarind seeds were collected from the local market and they were well washed, cleaned and dried in air. The testa and kernel in the seeds were estimated by the usual methods and were found to be 30°22 and 69°78 per cent respectively. The moisture in the kernel was estimated by heating a weighed amount of powdered kernel in an air oven at 40°C. for 2 hours and then at 80°C. for two hours and afterwards at 110°C. till a constant weight was obtained. The loss of weight represented moisture which was 10°2 per cent as an average of a few readings. For estimating pectin a number of experiments were carried out as stated above both for getting pectin as calcium pectate and methoxy group by Zeissel's method but there was no pectin.

Estimation of fixed oil

It was done by extracting the seed powder in a Soxhlet apparatus with petroleum ether (boiling 40°-60°C.). The fixed oil was found to be 6.8 per cent as an average of two estimations.

Estimation of albuminoids

They were determined by estimating the percentage of nitrogen by Kjeldahl's method in the

seed powder and multiplying the figure with the factor 6.4. Our result is 20.12 per cent as an average of three estimations while it is 15.4 per cent in Ghosh and Krishna's paper.

Reducing sugars

Reducing sugars were estimated by extracting a weighed quantity of the powdered kernel with water and titrating with standard Fehling's solution. The result was 2.8 per cent estimated in terms of glucose as an average of two estimations.

Crude fibres

Fibre was estimated according to the method given by Allen [1937]. It was found to be 2.4 per cent.

Ash

Estimated by incinerating a weighed quantity of the powdered kernel in a silica crucible until a constant weight was obtained. It was found to be 2.45 per cent.

Tannin

As no tannin could be detected in the powdered kernel by qualitative examination the question of estimation did not arise.

Determination of starch

Starch was estimated both by hydrolytic and non-hydrolytic methods. Hydrolysis with enzymes could be done only with malt-diastase. As Taka diastase which can hydrolyse both <- and B-starches easily to maltose and dextrin was not available in the market, malt diastase had to be employed. Fresh samples of this diastase were prepared in the laboratory as mentioned by Onslow [1929] and they differed in their activity as time for complete hydrolysis was quite different in each of the samples. O'Sullivan's method [Allen 1937] for the estimation of starch was used for diastatic hydrolysis but generally hydrolysis was not complete as B-starch is hydrolysed by malt diastase only under optimum pH condition which could not be obtained in every case. In some cases the hydrolysis was very quick and with a very active sample of diastase the hydrolysis was complete in an incubator running at 38°C. within 24 hours. Normally diastase required about three days for changing the starch completely to reducing sugars. Maltose was estimated by Fehling's solution and dextrin calculated from the rotation of the solution in a polarimeter. Results obtained by this method gave starch up to 50 per cent. The result was less by 14-15 per cent malt as diastase is not the proper enyzyme like Taka diastase for completely hydrolysing starch. There is another objection to using enyzymes as they introduce simultaneously bacterial contaminations and the necessity for a secondary conversion of the residual dextrins, which require considerable time. Allen [1937] also mentions that the results obtained by Sullivan's methods are 12-13 per cent lower than the actual value. The high content of albuminoids present in the seed was mainly responsible for the difficulties encountered in the estimation of starch. Filtering difficulties could only be overcome by filtering through an acid-washed layer of sand. Till the time starch and albuminoids were present it was most difficult to filter through a filter paper.

Acid hydrolysis [Scott, 1939] gave the exact result. In this method a few gm. of the seed powder (80-100 mash sieve) were taken in a round bottomed flask, 200 ml. of water and 5 ml. of glacial acetic acid were added. The flask was connected to a reflux condenser and the contents boiled for 1½ hours. Afterwards 15 ml. of concentrated Hcl were added and the mixture boiled under reflux for a certain period. Starch was changed to reducing sugars. After neutralizing the acid with sodium carbonate the reducing sugars were estimated either by direct titration with standard Fehling's solution or indirectly by Bertrand's method wherein the equivalent starch was easy to calculate as one ml. of N/30 KMnO<sub>4</sub> is equivalent to 0.0011 gm. of starch. In cases where the amount of glucose or reducing sugar was obtained by direct titration, the glucose figure was multiplied with 0.93 for getting the equivalent amount of starch. About acid hydrolysis a very important fact is necessary to mention. When the mixture was refluxed with the acid for half an hour the percentage of starch obtained was near about 47 per cent. After two hours heating with acid the result was 53-55 per cent. A number of experiments had to be carried out to get the maximum value. After about 8 hours of heating 65 per cent of starch was obtained. In some experiments the solution was heated for a greater time uptil a maximum of about 15 hours but the result did not exceed more than 65 per cent in these cases. Acid hydrolysis needed sufficient time for complete hydrolysis but it seemed to be the most accurate method.

In non-hydrolytic methods starch is dissolved in a solvent and then recovered and weighed or precipitated from the solvent in the form of a derivative. Starch dissolves in aquous NaOH and then precipitated by alcohol as starch is insoluble in alcoholic NaOH. Baumert and Bode's modifications as mentioned by Allen [1937] was used for this purpose. Another method used by Rask [1927, 1928, 1930] in America was used for this purpose. It is a very rapid method for

estimating starch in grains but it seems to fail in those cases where albuminoids are more than 12-13 per cent due to the filtration difficulties. principle used is the same alcoholic precipitation but all the solutions of starch in concentrated acid could not be filtered even under suction and for that purpose acid-washed sand had to be used. But as this did not give such a clear filtrate as that obtained by suction, the results were not consistent. Filtrate obtained either by suction or sand could not give a compact precipitate with alcohol. The precipitate was jelly like and could not be obtained in a compact form by any way. With the filtrates obtained by suction, results obtained were only up to 40 per cent. Some more work is needed on Rask's method for avoiding the various difficulties in this particular grain.

Modification of Rask's method as used by Herd and Kent-Jones [1934] as mentioned by Radley [1943] was also used wherein a centrifuge was employed every time, but here again the precipitate was of a jelly form and as no compact mass could be obtained the results were not consistent.

## Microscopical examination

Fine powder (100 mash) of the seeds was examined under a microscope and the starch granules were clearly seen. Micro-photographs were taken both with ordinary and with polarized light. (Plate VII, figs. 1 and 2) Starch granules are not only oval but of a few other shapes as well. A cross section of the seed was then prepared for examination by following an elaborate procedure consisting of softening the kernel, its dehydration and subsequent impregnation with wax according to well known methods used by botanists. This procedure was found necessary as on account of the hardness of the kernel and its very brittle nature, a section by hand microtome was not possible. Sections of about 5 \mu thickness were obtained by a mechanical  $microtome\ and\ a\ micro-photograph\ (Plate\ VII, fig.\ 3)$ of one such section is appended herewith which shows clearly the starch granules in cells. Another section was stained very lightly with iodine and micro-photographed (Plate VII, fig. 4). The dark particles, representing the starch granules on which iodine has acted forming the deep blue iodinestarch compound, are embedded in the cells. The magnification employed in all these cases was about 350 diameters.

#### SUMMARY

Tamarind seeds have been chemically analysed and, contrary to Ghosh and Krishna's result, it has now been definitely ascertained that they do not contain any pectin at all. The 64.4 per cent of pectin found by Ghosh and Krishna is really starch which they have mistaken for pectin. The complete analysis of the kernel of dry tamarind seeds as determined by the present authors is given below:

Starch	65·20 g	m.	per c	ent
Albuminoids	 20.12	,	,,	
Oil	 6.80	,,	,,	,,
Reducing sugar	 $2 \cdot 80$	,,	,,	,,
Crude fibre	 $2 \cdot 43$	,,	,,	,,
${f A}{f s}{f h}$	 $2 \cdot 45$	,,	,,	,,
Total	99.80			

From the above it is easily seen that tamarind seeds contain a large percentage of starch and albuminoids and as such would constitute, if properly processed and manufactured, a very important staple food comparing favourably in food value with wheat and maize.

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## STUDIES ON COMPOST

# INFLUENCE OF ADDITION OF SOIL ON CARBON AND NITROGEN ECONOMY **DURING COMPOSTING\***

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ONE of the special features of the Bangalore process of composting is the utilization of the agency of earth for preventing losses of nitrogen and obtaining increased yields of humified organic manure [Acharya, 1939, 1944]. The beneficial effect of an earth cover in the preparation of composts has been reported upon by Hutchinson [1916] and Ayyar [1928, 1933]; and earth, in the form of canal mud has been used for centuries by the Chinese in building up their composts [King, 1926]. But it must be admitted that the use of earth in the above cases appears to have been based mainly on empirical experience and observations on a field scale, rather than on any systematic experiments carried out to test the point under controlled laboratory conditions. Further, the influence of earth on carbon economy during composting does not appear to have received any attention so far.

It was therefore considered advisable to carry out comparative trials on a laboratory scale under controlled conditions so as to examine the influence of addition of earth on the composting process. The trials have now been repeated over three seasons and they have yielded similar results in all the three seasons. Typical results obtained in one batch of trials are reported in the present paper.

#### EXPERIMENTAL

The trials were carried out in shallow, wide, glazed jars arranged in three series: series A carrying mixed organic refuse only without the addition of earth, series B carrying 2000 gm. of garden soil only, without the addition of organic refuse and series C containing a mixture of the organic refuse with the soil. In series B and C, 2000 gm. of air-dry surface soil (red-loam) sieved through a 10 mesh sieve, were also added. In series C, the soil was uniformly mixed with the refuse, with the addition of the required quantity of water to keep the mass moist and in good tilth. Only water was added to series B.

\*Work carried out under the Compost Scheme of the Imperial Council of Agricultural Research

The mixed refuse added to each jar in series A and C consisted of 150 gm. ragi (Eleusine coracana) straw, 50 gm. coarse grass, 40 gm. hariali (Cynodon dactylon) grass, 50 gm. mixed weeds, 70 gm. hongay (Pongamia glabra) leaves and 75 gm. cattle dung. All the materials except the dung were air-dried and enough of each material for 56 jars was first gathered, cut into bits and mixed well, before weighing out aliquots for the jars. The required quantity of cattle dung was separately weighed out (on a fresh weight basis) for each jar and was made into an emulsion with water and mixed uniformly with the whole material in each

Each series consisted of 28 jars, four of which were removed, for purpose of analysis in quadruplicate, at the end of 1, 2, 4, 8, 12, 16 and 20 weeks of decomposition.

At the end of a definite period of aerobic fermentation four jars were taken out from each of series A, B and C; the contents were dried at 40°-50°C., then allowed to air-dry in the laboratory and weighed and powdered. The material from each jar was mixed well, spread on a sheet of paper and samples of about 20-50 gm., composed of small quantities taken from different portions of the mass, were taken in duplicate for analysis. Such samples were further pulverized and aliquots were taken for the determination of (a) moisture, (b) carbon, (c) total nitrogen, and (d) ash. Carbon was determined by the chromic oxidation method of Acharya [1936] and total nitrogen by the Gunning's modification of the Kjeldahl method [A.O.A.C., 1935].

The data obtained are presented in Tables I to V. Table I gives the analysis of the raw materials used for composting and Table II the total quantities of dry matter, carbon, nitrogen and ash taken initially in each of the series A, B and C. Tables III, IV and V give the total quantities of residual materials remaining in the jars after each interval of composting, their analyses and the total quantities of dry matter, carbon, nitrogen and ash recovered as well as the C/N ratio of the material at each stage.

Table I

Analysis of raw materials used

. Materials	Moisture in gm.		on dry ba	sis in gm.	Mater
. Interestinate	per cent	Carbon	Nitrogen	Ash	
Ragi straw Katcha grass Hariali grass Mixed weeds Hongay leaves Cattle dung Soil	4·24 5·12 6·21 3·64 5·96 80·6 2·1	39·48 40·11 41·05 26·56 41·07 6·52 0·712	0·639 0·851 1·772 1·371 2·117 0·307 0·0686	17·54 15·70 12·47 55·42 12·66 32·99 96·63	Hariali quin serie (compo without
6			of constituer		Total qu in serie (soil on

Carbon

59.22

20.06

Nitrogen

0.959

0.426

Dry matter

143.6

47.4

material

in gm.

150.0

50.0

Ragi straw

Katcha grass

TABLE II.—Contd.

Material	Quantity	Quantities of constituents in gm.						
- materiai	of fresh material in gm.	Dry matter	Carbon	Nitrogen	Ash			
Hariali grass Mixed weeds Hongay leaves Cattle dung  Total quantity in series A (compost without soil)	40·0 50·0 70·0 75·0 435·0	37·5 48·2 65·8 14·6	16·42 13·28 28·75 4·89 142·62	0·709 0·686 1·482 0·230 4·492	4·7 26·7 8·3 4·8			
Total quantity in series B (soil only)	2000 · 0	1958 · 0	13 · 94	1.343	1892.0			
Total quantity in series C (compost with soil)	2435.0	2315 · 1	156.56	5.835	1969 · 2			

Table III

Decomposition of mixed refuse without the addition of soil

Ash

25.2

- 00	Period of incubation	Air-dry weight	Total dry matter	Analysis on dry basis in gm. per cent		Total	C/N ratio			
	meansmon	ın gm.	in gm. in gm.	Carbon	Nitrogen	Ash	Carbon	Nitrogen	Ash	
C1	Initial		357 · 1	39.93	1.258	21 · 62	142.6	4.492	77 · 20	31.75
C2	1 week	342	308.9	38 · 29	1.249	24.31	118.3	3.859	$75 \cdot 12$	30.66
C3	2 weeks	303	274.5	36.82	1.394	$27 \cdot 61$	101.1	3.828	75.78	26.40
C4	4,,	264	237 · 2	33.98	1.565	32.56	80.6	3.713	77 · 24	21.71
C5	8 "	232	208.9	31.30	1.746	36.44	65.4	3.648	76.13	17.93
C6	12 ,,	211	190.5	$29 \cdot 34$	1.875	41.02	55.9	3.572	78.12	15.65
C7	16 ,,	202	183 · 4	27.59	1.900	42.45	50.6	3.484	77.86	14.52
C8	20 ,,	195	174.7	25.87	1.984	44.78	45.2	3.465	78 · 22	13.04

The figures given in Table III would show that when mixed refuse is decomposed without the addition of soil, there is considerable loss of nitrogen, amounting to nearly 23 per cent of the initial nitrogen content (Table VI). This loss in nitrogen is not revealed by the percentage analysis of the residual material (Table III), since the nitrogen content of the residue shows a progressive increase with period of decomposition; when however the nitrogen percentage is multiplied by the total dry matter present, the loss of nitrogen is found to be of a high order. A perusal of the figures for total nitrogen present in the residue at different periods of decomposition (Table III) would show that the loss occurs in two definite stages. Soon after the dung emulsion is added to the refuse and active fermentation starts, i.e. in the first week of decomposition there is rapid

loss of nitrogen, which in the present case amounts to about 14 per cent of the initial nitrogen content (Table VI). This is followed by a second stage of slower but progressive loss of nitrogen accompanying the progressive decomposition of the manure. It must be remembered that the refuse has been decomposed in this experiment aerobically.

The changes in total carbon recovered in the residues at different periods of decomposition follow a course roughly similar to the changes in nitrogen; but on account of the greater rate at which carbon is lost as compared to nitrogen, there is a progressive narrowing of the C/N ratio of the residue from about 32:1 to about 13:1.

The addition of earth to decomposing refuse exerts a profound influence on the carbon and nitrogen economy of the system, as shown by the data contained in Table V. In this case, there is no loss of nitrogen; on the other hand, there is a slight but significant fixation of atmospheric nitrogen taking place, the quantity so fixed in 20 weeks amounting to about 2 to  $2\frac{1}{2}$  per cent of the initial nitrogen content of the system. Deducting from the values given in Table V the control values for carbon and nitrogen present in the soil only at the end of 20 weeks incubation (Table IV) it is found that  $47.55\,\mathrm{gm}$ . of carbon and  $4.352\,\mathrm{gm}$ . of nitrogen are recovered from the original refuse in presence of soil, as compared to  $45.2\,\mathrm{gm}$ . of carbon and  $3.465\,\mathrm{gm}$ . of nitrogen recovered from

the same refuse when composted without the addition of soil. The difference is specially marked in the case of nitrogen, where the addition of soil has increased the recovery by nearly 25 per cent (Table VI).

The rate at which the C N ratio of the residue narrows (Tables III and V) is also greater in the refuse plus soil compost, indicating that humification proceeds more rapidly in presence of soil. Part of the narrower C/N ratios observed in the refuse plus soil composts, is no doubt attributable to the better conservation of nitrogen secured in the above composts.

TABLE IV
Changes in soil only

Serial No.	Period of	Air-dry weight	Dry matter	Analysis on dry	basis in gm.	Total quantiti in gm. p	
Seriai No.	incubation	in gm.	in gm.	Carbon	Nitrogen	Carbon	Nitrogen
S1 S2 S3 S4 S5 S6 S7 S8	Initial 1 week 2 weeks 4 " 8 " 12 " 16 " 20 "	2000 2000 2000 2000 1990 1990 1980 1980	1958 1956 1956 1954 1942 1942 1934	0·712 0·726 0·764 0·771 0·744 0·723 0·706 0·685	0.0686 0.0714 0.0742 0.0761 0.0762 0.0802 0.0817 0.0834	13·94 14·20 14·94 15·07 14·45 14·04 13·66 13·25	1·343 1·396 1·451 1·487 1·480 1·557 1·581 1·614

Table V

Decomposition of mixed refuse in presence of soil

Serial Period of No. Period of incubation weight in gm.			Analysis on dry matter in gm. per cent		Total quantities recovered in gm. per cent			C/N ratio		
	in gm.	Carbon	Nitrogen	Ash	Carbon	Nitrogen	Ash			
CS1 CS2 CS3 CS4 CS5 CS6 CS7	8 ,, 12 ,, 16 ,,	2390 2330 2285 2240 2200 2175 2150 2120	2315 2265 2225 2184 2149 2122 2098 2071	6·766 6·012 5·365 4·765 4·211 3·639 3·160 2·936	0·2522 0·2561 0·2628 0·2667 0·2721 0·2777 0·2839 0·2880	85·07 86·16 87·40 88·90 89·97 91·35 92·85 93·76	156·6 136·2 119·4 104·1 90·5 77·2 66·3 60·8	5·835 5·802 5·849 5·824 5·848 5·894 5·956 5·966	1969 1952 1945 1942 1933 1938 1948 1942	26·83 23·47 20·41 17·87 15·47 13·10 11·14 10·20

Table VI
Percentage recoveries of carbon and nitrogen

Decomposition in	Percentage recovery of carbon in gm.			Percentage recovery of nitrogen in gm.		
weeks	Refuse only	Soil only	Soil+Refuse	Refuse only	Soil only	Refuse + Soi
1. Initial 2. 1 week 3. 2 weeks 4. 4 ,, 5. 8 ,, 6. 12 ,, 7. 16 ,, 8. 20 ,,	100 · 00 82 · 96 70 · 89 56 · 52 45 · 86 39 · 20 35 · 49 31 · 70	100·0 101·9 107·2 108·1 103·6 100·7 97·97 95·04	100·00 86·96 76·25 66·46 57·79 49·29 42·34 38·83	100·00 85·92 85·23 82·66 81·23 79·52 77·57 77·14	100·0 104·0 108·0 110·8 110·3 116·0 117·7 120·1	100·00 99·40 100·2 99·79 100·2 101·0 102·1 102·2

## Discussion

The present experiment does not show in what form the nitrogen is lost when mixed refuse is decomposed aerobically with the addition of dung emulsion. Experiments are under way to examine this question in detail, but presumably a good portion of the loss occurs in the form of ammonia (subsequent data unpublished).

Shrikhande [Eden, 1939] who followed the decomposition of Gliricidia and tea leaves under controlled conditions found that in the case of Gliricidia, (C: N ratio near 11:1) more than 50 per cent of the initial nitrogen was lost as ammonia. In the case of tea leaves, however, (C: N ratio near 17:1), the loss amounted to about 9 per cent only, and of this only 0.6 per cent was recovered in the form of ammonia. Somewhat similar results were obtained by Jayaraman [1941] who followed the total losses of nitrogen, but did not measure the quantity of ammonia evolved (Table VII).

Table VII

Initial C/N ratio and percentage of initial nitrogen lost

Material	Initial C/N ratio	Percentage of initial nitrogen lost
<ol> <li>Dadap leaves</li> <li>Tea</li> <li>Grevillea</li> </ol>	12·3 19·1 36·7	50·6 14·0 2·3 (gain)

A great deal of work has been done on the rate of ammonification and nitrification of green manures [Waksman, 1938; Eden and Shrikhande, 1939] and the data obtained show that, in general, plant materials of C/N ratio narrower than 20:1 (of nitrogen content, say above 1.8 per cent on dry matter) tend to liberate ammonia on decomposition, whereas materials of C/N ratio wider than 20:1 (possessing a nitrogen content of say, lower than 1.8 per cent on dry basis) do not liberate ammonia, but on the other hand suffer from nitrogen 'hunger', as shown by their ability to fix added soluble nitrogen in the insoluble organic form [Hutchinson and Richards, 1921; Richards and Norman, 1931].

Applying the above criteria to the present experiment it would appear as though mixed refuse having an initial C/N ratio of 31·75:1 (Table III) should on decomposition show a tendency for fixation of additional nitrogen and certainly for no appreciable loss of nitrogen. The actual loss observed, however, is considerable, amounting to over 20 per cent of the initial nitrogen contained in the refuse.

The above loss is explainable on the basis that the limits of C/N ratios indicated by previous workers as controlling nitrogen losses, apply only to single plant materials of a uniform type, and not to mixtures of different plant materials. In the case of such mixed refuse, the over-all C/N ratio of the mixture may not possibly give any indication of the behaviour of the individual constituents. It is probable that microbial attack may start vigorously on such of the components of the mixture as possess narrow C/N ratios, while the more resistant components of wider C/N ratios may remain unattacked in the early stages. Thus, the ammonia that may be liberated in the earlier stages from the components of narrow C/N ratios may be lost in part before it is used up by the micro-organisms for the decomposition of the components of wider C/N ratios. In the present experiments, the over-all C/N ratio of the mixed refuse in series A is 31.75, but some of the constituents such as hongay leaves, mixed weeds and hariali grass possess narrow C/N ratios of 19.40, 19.37 and 23.16 respectively, while other components such as ragi (Eleusine coracana) straw and katcha grass possess wide C/N ratios of 61.79 and 47.13 respectively; and the hypothesis set out above would satisfactorily explain why there is a loss of over 20 per cent of the nitrogen from the above mixture.

The beneficial effect of earth in preventing nitrogen losses could be attributed to the following factors:

(1) The presence of soil colloids, which might absorb and retain the ammonia evolved.

(2) The influence of the type of microbial population present in the soil, which might utilize and fix up the ammonia evolved more rapidly than the microbial population present in decomposing plant refuse. Some recent work of Mr Pillai in this laboratory (unpublished data) has shown the important role played by protozoa in the activated sludge process, in improving the nitrogen content of sludge by fixation from sewage. The protozoa not only control the rate of liberation of ammonia by bringing down bacterial numbers, but also serve to fix rapidly in their own bodies the ammonia that is evolved in the system. It is possible that a similar role may be played by protozoa present in the soil added to compost, thus serving to minimize the over-all losses of nitrogen considerably.

(3) A part of the nitrogen loss from the refuse, may be masked by a simultaneous fixation of atmospheric nitrogen effected by the microorganisms present in the added soil. A reference to Tables IV and V would show that 2000 gm. of soil when incubated alone fixed 0.271 gm. of nitrogen from the air, but a mixture of the above quantity of soil with plant refuse fixed only 0.131

gm. of nitrogen. The difference might probably represent the amount of nitrogen lost from the organic refuse, since the nitrogen fixing power of soils has, in general, been found to be improved by the addition of refuse of comparatively wide C/N ratios.

In the absence of more definite information and data, it is difficult to assign relative values for the importance of the above three factors. Factors (2) and (3) mentioned above would be served by mixing the refuse with soil or by interspersing thin layers of garden earth between layers of refuse in compost-making. Factor (1) mentioned above, however, would require that the compost should be covered over with a layer of earth on top. In the Bangalore process of compost making, both these methods of using earth are adopted, viz. thin layers of earth in between layers of refuse and an earth plaster on top.

The beneficial effect of earth on carbon economy of compost, has got a practical bearing in relation to the quantity of organic manure that is finally obtained. The amount of organic manure recovered (total manure less earth and ash) is roughly proportional to the amount of carbon recovered; and since the aim of composting is to secure as high a recovery of organic manure as possible (of the proper quality) from the original refuse, the recovery of carbon, under such conditions, could be taken to be a measure of the efficiency of the

composting process in this respect.

Since the manure produced by a satisfactory system of compost making generally reaches a C/N level near 10:1, the amount of nitrogen that is conserved in the composting process would directly control the quantity of carbon conserved and thus, indirectly, the total quantity of organic manure obtained. Thus, in two systems of compost-making both of which yield finally a manure of C/N ratio near 10:1, if the total nitrogen recovery in one system is only 40-50 per cent, and in the other 90-100 per cent, it is clear that the former method of composting would yield only about half the quantity of organic manure yielded by the latter method. The importance of adopting satisfactory methods of preventing nitrogen loss during composting, in order to obtain the highest recoveries of manure possible, is therefore evident.

It is sometimes stated that the losses of nitrogen that take place during aerobic composting would be made up by subsequent fixation from the air, when the material decomposes further; and thus the final efficiency would be the same in all cases. But the experimental data already presented by one of the writers [Acharya, 1939, 1940] would show that in cases where the initial C/N ratio of the refuse mixture is about 30:1 or narrower in a period of 20 weeks. The loss appeared to

and some of the refuse constituents are rich in nitrogen, e.g. night-soil or urine, the quantity of organic manure finally obtained is considerably greater in the cases where nitrogen loss is avoided. The work of Dhar and co-workers [1937] has shown that cellulosic materials fix from 5 to 10 mg. of nitrogen per gram of carbon decomposed. The difference between the nitrogen loss-cum-fixation system and the nitrogen-conservation-system is brought out schematically in Table VIII and is found to be in favour of the latter system. This is due to the fact that the quantity of nitrogen gained with difficulty by fixation from the air is only a fraction of what is easily lost by adopting defective systems of composting.

TABLE VIII

Carbon and nitrogen economy during composting

100 lb. dry matter of refuse plus urine or night-soil

C=30 per cent, N=1 per cent C:N ratio 30:1

		U:N Taulo	
Constituent	s	Aerobic decomposition with turnings (approximate figures)	Bangalore process (approxi- mate figures)
Initially present	Carbon Nitrogen	30 lb. 1 lb.	30 lb. 1 lb.
Present after 1st stage of active decomposition for 2 weeks	$\begin{cases} \text{Carbon} \\ \text{Nitrogen} \end{cases}$	20 lb. 0·5 lb. (loss 0·5 lb. N)	25 lb. 1 lb.
Present after 2nd stage of 3 months' decomposition including N fixation from air	Carbon Nitrogen	7 lb. 0·5 lb. +0·13 lb. N fixed from air by 13 lb. of C decomposed Total=0·63 lb. N	11 lb. 1 lb.
Final C <sub>1</sub> N ratio		10.1	11

## ACKNOWLEDGEMENT

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#### SUMMARY

1. Mixed refuse containing weeds, leaves, grass, straw and cattle dung, having a C/N ratio near 32:1, was composted aerobically in jars, under controlled conditions, with and without the addition of garden earth.

2. The refuse composted without the addition of earth lost nearly 23 per cent of its initial nitrogen occur in two stages: there was an initial period of rapid loss, which covered the first week of decomposition; this was followed by a longer period of slow and steady loss, along with the progressive decomposition of the refuse.

The loss of nitrogen was overcome by the addition of earth to the compost mass, the over-all result being a slight gain in total nitrogen, due to fixation from the air.

4. Parallel with the increased conservation of nitrogen secured by addition of soil to decomposing refuse, there was an increased recovery of carbon and of organic matter in the final manure.

5. The C/N ratio of the compost narrowed down quicker in cases where soil had been added to the refuse.

6. Probable explanations are offered for: (a) the considerable losses of nitrogen taking place from mixed refuse of C/N ratios wider than 30:1, (b) the beneficial effect of earth in controlling such nitrogen losses; and (c) the relation between recovery of carbon and of organic manure on the one hand and of nitrogen economy during composting on the other.

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# A STUDY ON THE ESTIMATION OF THE YIELD OF WHEAT BY SAMPLING\*

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(Received for publication on 5 April 1945) (With five text-figures)

In any attempt at estimation of crop yields by sampling, the fixing up of an appropriate size and shape of the sampling unit and of the percentage area to be sampled are the essential prelimi-If the percentage of total population sampled remains the same, the degree of accuracy attained will depend mainly on the size and shape of the sampling unit and the distribution of these units in the general population. The actual shape and size of the sampling unit will be largely governed by the order of magnitude of the cropcutting experiments. Thus the administrator who is interested in the crop-outturn of large areas has to deal in terms of districts and hence the unit used by him is bound to be different from that employed by an experimentalist desiring to estimate the yield of an experimental plot. The exact sampling technique, the arrangements of the ultimate units, the number of units to be sampled and the distribution of these in the population, will differ in each case as these will depend upon

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†Mr. A. K. Mallik assisted by Mr. Satakopan conducted this field experiment. Mr. Satakopan supervised the statistical computations.

the nature and magnitude of the variability involved. But it should, however, be possible in practice to evolve for any crop, a hierarchy of sampling techniques to deal with cases ranging from individual plants to the district crop as units and covering all intermediate cases. In designing an experiment to give a complete solution of the problem of any one crop, it is possible, at one time to handle only two or three of the stages in the hierarchy.

The present paper deals with one such experiment designed to give information on the most suitable sampling technique for estimating the yield of wheat, variety A115, conducted in March, 1939, at the Government Experimental Farm, Powerkheda, Hoshangabad, C.P., with the kind co-operation of the Deputy Director of Agriculture, Northern Circle, C.P. The experiment is first described and then the important results obtained are discussed.

#### MATERIAL AND METHOD

A wheat field, ready for havesting, from the non-experimental area of the farm was chosen. A square plot, 4/10 of an acre, i.e. 132 ft.  $\times 132$  ft. was marked out for the experiment. This area

was harvested completely square yard by square yard and the yields of grain and straw were recorded separately from each unit (i.e.  $3 \, \mathrm{ft.} \times 3 \, \mathrm{ft.}$ ). As the marking of the whole plot into square yards was inconvenient for operations, the plot was sub-divided into 16 sub-plots of 33 ft. ×33 ft. each (1/40 of an acre), leaving a 3 ft. path along two edges of each sub-plot for trampling. The remaining area of 30 ft. ×30 ft. from each sub-plot was first marked out into 100 sq. yd. by strings tied across the sub-plot to pegs fixed at the boundaries of the sub-plot. 1600 sq. yd. were thus separately cut and the grain threshed by hand by labourers. The yields of grain and straw were recorded, correct to 1/16 of an ounce. The plants were cut with knives, and as it was not possible to ensure uniformity among the labourers in the method of cutting, the yields of straw recorded were not strictly comparable. Records of straw vields obtained from the experiment were therefore rejected and only grain yields were taken into consideration.

A plan of the whole experimental plot is shown in Fig. 1. As the orientation of the lines of plants has a bearing on the discussion of the results, the general direction of the rows is also indicated in the plan. The rows were roughly parallel to AB in Fig. 1.

1	5	9	13
2	s	10	14
3	A1 A.B. R.	11	15
4	8	12	16

Fig. 1. Field, sub-plots and constituent square yards

It may be mentioned that the yield figures for grain do not represent the yields from 1600 contiguous square yards but suffer from certain discontinuities due to the fact that one yard of the crop was rejected around each sub-plot as this was being constantly trampled during operations. But this discontinuity is not expected to affect the result of the analysis to any serious extent as it is likely to vitiate only the variances between sub-plots to a small extent.

## DISCUSSION OF THE RESULTS

Frequency distribution of the yields

In the first place the frequency distribution of the yields was studied. The frequency distribution of the yield from 1600 sq. yd. is shown in Table I and graphed in Fig. 2. The following distribution constants may be of interest in respect of the 1600 yields as a whole.

Table I
Frequency distribution of the yield of grain

Weight of grain yield in 1/16 oz.	Number of square yards	Weight of grain yield in 1/16 oz.	Number of square yards
12–13	8	32–33	116
14-15	18	34-35	85
16-17	62	36-37	45
18-19	94	38-39	28
20-21	159	40-41	32
22-23	175	42-43	15
24-25	184	44-45	6
26-27	176	46-47	3
28-29	224	48-49	3
30-31	165	50-51	
		52–53	2
			1600

g<sub>1</sub> which is a measure of asymmetry and which has a zero value for the normal distribution is significant for this distribution, being nearly 24 times its standard error. This indicates that the frequency is asymmetric. The value of g<sub>2</sub> is not significant (judged from the 5 per cent level) which indicates that the curve is not materially different from a normal curve in respect of kurtosis, i.e. flatness of the top.

 $<sup>*</sup>g_1$  and  $g_2$  are constants which measure the asymmetry and kurtosis of a frequency distribution curve. Their values are zero for a normal distribution. [Fisher, 1936].

Weight in Ounces

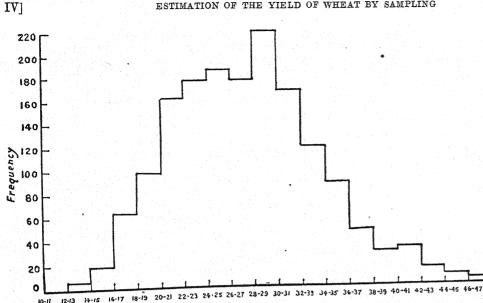


Fig. 2. Frequency distribution of yields of individual square yards

## Analysis of variance

In order to estimate the variances between units of different sizes and shapes, the 1600 sq. yd. were grouped consecutively into composite units consisting of 2, 3, 4, ........50 contiguous square yards, and the analysis of variance was worked out for each grouping. For example, for the contiguous unit of 2 sq. yd., the 100 sq. yd. in each sub-plot were divided into 50 units of two square yards each and the allocation of degrees of freedom in the analysis of variance was as follows:

아들아가 되었다면 말하다 하시는데, 맛없다	D.F.
Sub-plots	15
Between composite units	784
Within units	800
[[생][[[[생][[]]]]	
	1599

TABLE II Different sizes and shapes of composite units studied

Serial No.	Size of composite unit	Arrange- ment of square yards in the unit*	Remarks
1	2	2h	All sq. yards used in each
2	2	2⊽	đo.
3	3	3h	The last column at the right end omitted from each plot
4	3	3v	The last row at the bottom of each plot omitted
5	4	4h	The last two columns omitted

# TABLE II-Contd.

Serial No.	Size of composite unit	Arrange- ment of square yards in the unit*	Remarks
6	4	$2\mathrm{h}\!\times\!2\mathrm{v}$	All units used in each plot
7	4	4v	The last two rows omitted in each plot
_		5h	All units used
8	5	5v	do.
9	5 6	$3h \times 2v$	Same as item 3 above
10 11	6	$3v \times 2h$	Same as item 4 above
12	8	$4h \times 2v$	Same as item 5 above
13	8	$4v \times 2h$	Same as item 6 above
13	9	$3h \times 3v$	The last column and last
14	-	OH AU.	row omitted from each plot
15	10	$5h \times 2v$	All units used
16	10	$5v \times 2h$	do.
17 *	12	4h×3v	The last two columns and last row omitted
18	12	<b>4</b> ∇×3h	The last column and last two rows omitted
19	15	$5h \times 3v$	The last row omitted
20	15	$5v \times 3h$	The last column omitted
21	16	4h×4v	The last two rows and last two columns omitted
22	20	$5h \times 4v$	The last two rows omitted
23	20	$5v \times 4h$	The last two columns omitted
24	25	$5h \times 5v$	All units used
25	50	$10h\times5v$	do.
26	50	$10v \times 5h$	do.

\*v= along CD in Fig. 1. and h= along AB in Fig. 1.

These have a reference to the directions on the plan. of Fig. 1. For example,  $5h\times 2v$  means a composite unit of 10 sq. yd. made of 5 yards along AB and 2 yards along CD in Fig. 1.

It should be mentioned here that as the sub-plots have been kept separately throughout the analysis it was not possible to utilize all the square yards from each plot in forming composite units of certain sizes. The available number of composite units of the requird sizes was taken from each sub-plot, the remaining square yards being omitted. The peculiarity in each case in this respect is indicated in the remarks column of Table II. The differences in the total variance and variances due to sub-plots seen in Table III arise also out of this fact, that all the units could not be utilized in forming different sizes of composite units.

The variance between samples in relation to the orientation of the rows

Since the crop was sown with a three-tyned drill, it was considered that the orientation of the lines of the crop will have a bearing on the variances between composite units (i.e. samples) of different

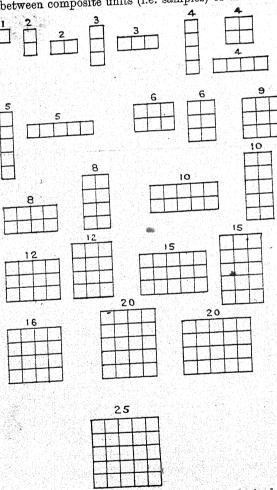


Fig. 3. Size and shape of sampling units obtained by different combinations of square yards

shapes. Each size of composite unit has therefore been studied in two or more alternative arrangements of square yards. Thus a composite unit of 4 sq. yd. has been taken first by combining four contiguous square yards along AB (Fig. 1), secondly by combining four contiguous square yards along CD (Fig. 1), and thirdly by taking a compact block of four square yards, two yards by two yards. The different types of composite units considered for each size are illustrated by Fig. 3.

Table III shows the analyses of variance for different shapes and sizes of composite units. It will be seen from column 3 of Table III that for the same size of the composite unit the arrangement of square yards with an elongation along AB gives a greater variance between composite units than the arrangement elongated along CD. Fig. 4 also indicates this clearly. Considering that the rows were more or less parallel to AB (Fig. 1) it appears that there is greater variability between the rows of plants than within the row. This is also confirmed as it should be evident, by the smaller, "within units variance" for the AB elongated units than for the CD elongated units. It would therefore appear that in dealing with the crop it is always better to take a sample elongated in a direction at right angles to the plant rows to keep the sampling variance low.

Table III

Analysis of variance of different shapes and sizes
of composite units

Size and shape	Variances between sub-plots	Variance between composite units	Variance within units	Total variance
01	639.3	46.3	20.7	39.0
2h 2v	639.3	44.1	22.8	39.0
2v 3h	584.2	49.5	25.4	39.0
	555.2	48.6	25.7	38.6
3₹	491.1	68.6	22.2	38.7
4h	476.5	57.0	26.5	39.0
4v	639.3	65.3	23.1	39.0
$2h\times2v$	639.3	74.3	23.6	39.0
5h	639.3	55.7	28.0	39.0
5⊽	584 · 2	82.6	24.0	39.0
$3h \times 2v$	555.2	73.3	25.7	38.6
$3v \times 2h$	491.1	106.3	24.0	38.7
$4\text{h} \times 2\text{v}$	476.7	86.8	26.9	39.0
$4\sqrt{2h}$	512.7	96.9	26.0	38.7
$3h \times 3v$	639.3	113.7	25.3	39.0
$5h \times 2v$	639.3	84.3	28-2	39.0
$5v \times 2h$	431.9	126.5	26.2	38.5
$4h \times 3v$	437.1	115.1	27.9	39.3
$4v \times 3h$	555.2	136.6	27.0	38.6
$5h \times 3v$	644.2	113.0	28.5	39.6
$5v \times 3h$	366.3	153.4	28.3	39.1
$4h \times 4v$	476.5	172.8	28.3	39.0
$5\mathrm{h}\!\times\!4\mathrm{v}$		145.6	28.9	38.7
$5v \times 4h$	491.1	152.5	29.6	39.0
$5h \times 5v$	639.3	277.6	30.8	39.0
$10h \times 5v$ $10v \times 5h$	639.3	138-5	32.3	39.0

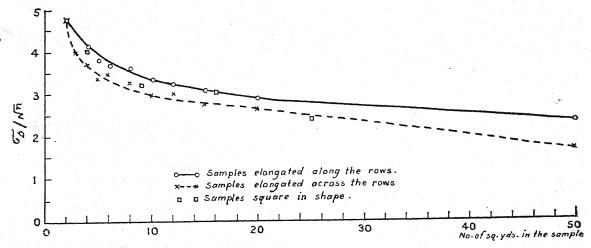


Fig. 4. The standard error of mean yield per square yard  $(\sigma_s)/(\sqrt{n})$  in relation to the size and shape of the sample

The optimum size and shape of the sample

Table IV gives the standard error between composite units (square root of variance between units) and also the standard error of mean yield for each sample  $\sigma_s / \sqrt{n}$  where n is the number of square yards in each sample. The values of the standard errors of the mean yields have been plotted against the size of the unit in Fig. 4 for both the AB and CD elongated samples. The point representing compact structures of composite units are shown separately. It is seen from Fig. 4 that  $\frac{\sigma_s}{\sqrt{n}}$  decreases as the size of the sample increases, showing that the variability of the mean yield decreases with size of unit. But the decrease is greater for small units and as the size increases the decrease is less. In fact the slope of the curves for samples larger than 15 units is considerably less than that of the part of the curve representing smaller units. It appears therefore that a sample comprised of 12 to 15 square yards arranged in such a way that 4 or 5 rows are included in the sample, i.e.

4 square yards along CD and 3 sq. yards along AB or 5 square yards along CD and 3 sq. yards along AB, is a suitable unit for sampling operations on the crop.

Estimation of yield by random sampling

Estimation of the sub-plot yield by choosing samples at random from each sub-plot was next considered. Four square yards were selected at random from each sub-plot and the sub-plot yields estimated from these. Five square yards were then selected at random from each sub-plot

and the yield of each sub-plot was estimated therefrom. Such experiments were repeated increasing the number of square yards up to 50. In the last

Table IV

Standard errors from variances in Table III together with standard error of mean yields for each composite unit

Size and shape of	Sub-plot	Composite	Within	$\sigma_s / \sqrt{n}$
composite units	error	units $\sigma_{\overline{s}}$	units	
2h×1v	25 · 28	6.801	4.545	4.81
$2v \times 1h$	25.28	6.639	4.774	4.69
$3h \times 1v$	$24 \cdot 17$	7.036	5.038	4.06
$3v \times 1h$	23.56	6.971	5.068	4.02
$4h \times 1v$	22.16	8.282	4.711	4.14
$2h \times 2v$	25.28	8.083	4.805	4.04
$4v \times 1h$	21.85	7.551	5.145	3.77
5h×lv	25.28	8.618	4.859	3.85
$5v \times lh$	25.28	7-467	5.292	3.34
$3h \times 2v$	24.17	9.090	4.901	3.71
$3v \times 2h$	23.56	8.561	5.065	3.50
$4h \times 2v$	22.16	10.309	4.897	3.64
$4v \times 2h$	21.83	9.316	5.185	3 · 29
$3h \times 3v$	22.64	9.844	5.101	3.28
$5h \times 2v$	25.28	10.661	5.030	3.37
$5v \times 2h$	25.28	9.183	5.313	2.90
$4h \times 3v$	20.78	11.25	5.12	3 · 25
$4v \times 3h$	20.91	10.73	5.29	3.10
$5h \times 3v$	23.56	11.69	5.20	3.11
$5v \times 3h$	25.38	10.63	5.34	2.74
$4h \times 4v$	19.14	12.38	5.32	3.09
$5h\times4v$	21.83	13.15	5.32	2.94
$5v \times 4h$	22.16	12.07	5.38	2.70
$5h \times 5v$	$25 \cdot 28$	12.35	5.44	2.47
$10h \times 5v$	25.28	11.77	5.68	2.36
$10v \times 5h$	25.28	16.66	5.55	1.67

case half of each sub-plot was sampled out and the yield of whole sub-plot was estimated therefrom.

Apart from estimating the plot yields the percentage information obtained for each size of the random sample was calculated by the formula

Holom sample was calculated by P=100(I-L) where  $L=\frac{n-2}{n}\left(1-\frac{k}{\bar{h}}\right)\frac{B}{A}$ ,

where— P =Percentage information obtained

L =Loss of information

k/h =Proportion of plot sampled B =Variance between samples

A =Variance between sub-plots
n =Number of sub-plots

For theoretical considerations behind this formula a reference may be made to Yates and Zacopanay [1935].

Table V

Results of random sampling of different percentages from sub-plots

Number of	Standa	rd error	ntage ation	Mean yield per square yard as estimated by sampling in 1/16 oz.	
square yards sampled from each sub-plot	Plot	Within plot	Percentage information obtained		
4	6.66	5.72	38	26.98	
5	7.44	5.91	47	25.30	
6	$7 \cdot \hat{27}$	6.00	44	27.47	
7	$11.\overline{27}$	5.77	79	26.83	
8	10.58	5.46	79	26.77	
	9.88	5.83	73	27.16	
$\begin{array}{c} 10 \\ 12 \end{array}$	11.67	5.90	80	27.49	
	11.14	6.11	77	26.78	
14	10.99	5.58	81	26.80	
16	12.90	5.85	86	26.46	
20	13.92	5.66	89	26.79	
25	13 92	5.65	90	26.67	
30	16.64	5.77	94	26.62	
· 40 50	18.10	5.93	95	26.67	

Actual mean yield per square yard in 1/16 oz.=26.71

Table V gives the estimated and the actual mean yield per square yard for the different sizes of sample together with the percentage information obtained. It is seen that sampling 12 to 15 per cent of the crop gives a percentage information of the order of 80. The relationship between the percentages of total population sampled and the information obtained is shown graphically in Fig. 5. It will be seen that the increase in the percentage information obtained is rapid till the per cent population sampled reaches a value of about 12 to 15 per cent; further increase in percentage sampled increases the percentage information at a slower rate. Here again it would appear that sampling about 12 to 15 per cent of the total population will give a fairly good estimation of sub-plot yields.

Random sampling with composite units

Next, the question whether composite units can be used for sampling and if so, what will be the best size and shape of the units to be taken was studied. Table VI gives the results of the analyses for three percentages of sampling 12, 15 and 16 per cent. For each percentage of sampling, composite units of different sizes have been taken and the percentage information obtained by the sampling has been worked out. It will be seen that the following sampling techniques give over eighty per cent of the information.

Size of composite units	Number of composite units
1. 4 contiguous square yards a the rows along CD	cross 4
2. 2 sq. vd. along CD	8

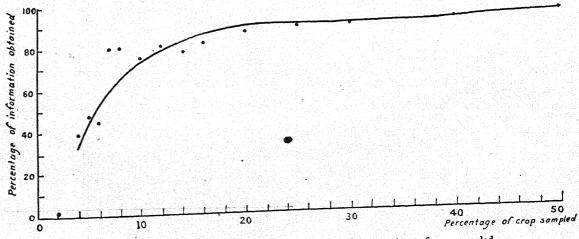


Fig. 5. Percentage information obtained in relation to the percentage of crop sampled

TABLE VI

Results of random sampling with composite units for 12, 15 and 16 per cent sampling

Composition	No. of units selected at random	Standard error* per composite unit		Percentage information	Percentage sampled to total yield
of composite unit	No. c selec rai	Plot	Within plots	Perc info	Percel sample total
2v 2h 3v 3v 3h 4v 4h 2v×2h 6h 6h 3v×2h 3h×2v	6 6 4 4 3 3 3 2 2 2 2	16·75 13·81 21·91 22·83 18·66 26·94 27·71 22·21 23·04 27·43 25·34	8.95 8.48 14.37 13.39 13.87 16.33 24.81 21.62 20.94 16.91 18.25	78 71 68 74 58 72 38 28 37 71 60	11.95 12.02 12.12 11.79 12.06 12.07 11.51 11.78 11.63 11.95 12.04
15 per cent sampling 2 A 2 2 4 2 4 4 4 4 4 4 4 4 4 4 4 4 4 4	5 5 3 3	22·06 21·88 20·58 24·59	12·26 11·68 18·73 17·96	77 79 39 61	14·79 14·64 15·23 15·05
2v 2h 2h 3v 3sh 3sh	8 8 4 4 2 2	$   \begin{array}{c}     17 \cdot 54 \\     14 \cdot 22 \\     27 \cdot 71 \\     27 \cdot 34 \\     41 \cdot 59 \\     35 \cdot 30   \end{array} $	8·37 9·41 12·64 15·03 28·11 29·02	83 68 85 78 67 51	16·24 15·61 16·10 15·96 16·08 15·67

\*The standard errors are based on the analysis of variance of total yield of each composite unit and not reduced to per square yard. Hence the standard errors for different sizes of composite units are not comparable without reduction to the same size.

The second arrangement would involve double the number of units to deal with and so the first is preferable from the point of view of practicability. It would thus appear that for estimating yields by random sampling, four units of 4 sq. yd. each (4 yd.×1 yd. the length being at right angles to the rows) from each sub-plot will be most suitable.

#### SUMMARY

The present paper deals with a complete harvesting experiment with the object of finding out the most appropriate size and shape of sample to be used for the estimation of the yield of wheat by sampling. The procedure adopted is described in detail. Statistical analysis of the data shows:

1. Samples elongated across the rows are less variable than those elongated along the rows.

2. The optimum percentage of total population sampled is about 16 per cent. There is no material gain in information by sampling more than 16 per cent of the crop.

3. Samples composed of 3 yd. along the rows and 4 or 5 yd. across the rows are the most suitable.

### ACKNOWLEDGEMENTS

Our best thanks are due to Dr L. A. Ramdas, Agricultural Meteorologist for suggesting the problem and for guidance in the preparation of this paper. Thanks are also due to Dr R. J. Kalamkar, Deputy Director of Agriculture and the staff at Powerkheda Experimental Farm, Northern Circle, C.P., for their kind co-operation.

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# PLANT QUARANTINE NOTIFICATION

Notice No. 1 of 1945

THE following Quarantine Regulations have been received in the Imperial Council of Agricultural Research. Those interested are advised to apply to the Secretary, Imperial Council of Agricultural Research, New Delhi.

(1) Plant Quarantine Import Restrictions of the territory of Southern Rhodesia—B.E.P.Q.446, Supplement No. 1 dated the 3rd November, 1944, issued

by the United States Department of Agriculture.
(2) Plant Quarantine Import Restrictions of the Republic of Peru—P.Q.C.A.310, Supplement No. 6, Revised, dated the 16th November 1944, issued by the United States Department of Agriculture.

(3) Service and Regulatory Announcements April-June 1944, B.E.P.Q.159, issued by the United States Department of Agriculture.

# ORIGINAL ARTICLES

# OBSERVATIONS ON THE INFLUENCE OF NITRATE, AMMONIUM AND IRON ON THE GROWTH AND NUTRITION OF THE RICE PLANT

By R. D. Asana, Plant Physiologist, Central Sugarcane Research Station, Pusa

(Received for publication on 25 November 1944)

(With Plate VIII and two text-figures)

THERE appears to be a considerable difference of opinion on the ralative merits of nitrate and ammonium ions as sources of nitrogen for the rice plant. As the literature on the subject is very extensive no attempt is made to review it; recent investigations which bear directly on the observations re-

ported in this paper are alone considered.

Espino and Estioko [1931], Dastur and Malkani [1933] and Dastur and Pirzada [1934] find a mixture of both forms of nitrogen more satisfactory than either used alone. Thelin and Beaumont [1934] find that in a mixture of nitrate and ammonium nitrogen relatively greater proportion of the former is more satisfactory than the reverse. Gericke [1930] obtained satisfactory growth with nitrate alone as the source of nitrogen in a complete culture solution. Willis and Carrero [1923] from a comparative study of the influence of nitrate and ammonium in soil cultures conclude that the nitrogen of calcium nitrate may be as suitable to the rice plant as that of ammonium in which the reaction of the unassimilated residue does not interfere with the absorption and utilization of iron.

It appears that the earlier work of Gile and Carrero [1920] and Willis and Carrero [1923] did not receive the attention it deserved. They were the first, so far as the writer is aware, to point out the relation of iron to nitrate and ammonium ions. Gines [1930] from Espino's laboratory, did not compare the influence of iron salts on nitrate and ammonium cultures used separately although that is the important point as already made clear by Willis and Carrero [1923]. Dastur and John [1938] investigated the influence of different salts of iron and different pH on the growth of rice seedlings in Knop's solution as modified by Tottingham. Seedlings 14 days old from the time of sowing were used and the experiment lasted for a fortnight. There was no difference between cultures with ferrous sulphate and without iron. The lowest dry weight at  $p \to 3.6$  was 233.3 mg. (per 30 plants) as against the highest obtained at  $p \to 7.0$ , viz. 261.0 mg. Thus in a fortnight only 28 mg. dry weight (per 30 plants) was added which appears to be very low. These workers make no mention of the light and temperature conditions prevailing at the time of the experiment.

Dastur and Malkani [1933] did not repeat their absorption experiments during the 8 to 18 day stages, as well as those on the influence of different concentrations with complete culture solutions; also they did not collect growth or dry matter data from these experiments. Dastur and Pirzada [1934] obtained growth data from the transplantation stage onwards but they applied fertilizers directly to the soil which unfortunately introduced another complication.

It was the aim of this investigation to study more fully the interaction of iron, nitrate and ammonium ions on the growth and nutrition of rice in its early stage, as this aspect had not received enough attention so far. The writer is conscious of the limitations of the data presented but as there is no possibility of the work being continued the observations have been brought together in the hope of calling attention of other workers to this aspect of the

problem.

#### EXPERIMENTAL

Seeds of a pure strain of rice (lowland variety Co 10) obtained from the Coimbatore Paddy Breeding Station were used throughout. The seeds were steeped in water for about six hours and then kept between moist filter paper at room temperature for two or three days until the radicale attained a length of 3-4 mm. The germinated seeds were then transferred to perforated cork mats floating on tap water in glazed porcelain beakers; the seedlings were used for experiment when the shoot had grown about 4 cm. long. The time between soaking the seeds and the shoots attaining the length of about 4 cm. varied from six to nine days depending upon the temperature prevailing at the time of the year; these observations were carefully recorded.

For experiment the plants were kept outside in a wire netting cage (mesh about \( \frac{3}{4} \) sq. in.) with a glass roof, which fairly approximated to a green house. Thus the experiments were carried out under natural conditions of light and temperature. Daily maximum and minimum temperature records were kept and the records of hours of bright sunshine were obtained from the local meteorological station about a mile away. Although topographical variations are bound to occur these records throw some light on the differences in results obtained at different times and these will be considered in the appropriate place.

The culture vessel consisted of a tall glass beaker (resistance glass) of one litre capacity, made opaque to light by thick paper wrappings. On top of the beaker rested a 'urolite' disc with seven perforations to receive the plants, one in each, which were

held in position by cotton-wool plugs.

A few experiments were carried out in diffuse light (i.e. not direct sunlight and under shade); this light was, however, not enough for good growth of the plants as results of Experiment 1 show. The culture vessel in these experiments consisted of a glazed porcelain pot about 7 in diameter and 600 c.c. capacity. These experiments are referred to as 'diffuse light experiments' (Experiments 1

In all experiments complete culture solutions were used. The culture solutions with ammonium and nitrate ions as the only sources of nitrogen will henceforth be referred to, for the sake of brevity, as NH<sub>4</sub> and NO<sub>3</sub> cultures respectively. Glass distilled water was always used for making up the cultures. The NH<sub>4</sub> culture had the composition: KH<sub>2</sub>PO<sub>4</sub>, MgSO<sub>4</sub>, CaSO<sub>4</sub>, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and FePO<sub>4</sub> and the NO<sub>3</sub> culture: KH<sub>2</sub>PO<sub>4</sub>, MgSO<sub>4</sub>, ·Ca(NO<sub>3</sub>)<sub>2</sub> and FeSO<sub>4</sub>. The concentration of each nutrient in p.p.m. was: K<sub>2</sub>O, 14; P<sub>2</sub>O<sub>5</sub>, 21; N, 14; Ca, 19; Mg, 24; and Fe, 8.

# Methods of chemical analysis

Drying. The fresh plant material was first dried for about half an hour at about 90°C. and then between 65° and 70°C. for about 27 hours, when the

dry weight was found to be constant.

Nitrogen was estimated by the micro-Kjeldahl method as adapted by Parnas and Wagner and described by Pregl [1924]. Nitrate was reduced by reduced iron. Calcium from the plant ash was precipitated as Ca-oxalate by ammonium oxalate at pH 4.5 and the precipitate was then titrated against KMnO<sub>4</sub>. For manganese, all interfering ions including chloride were removed and the magenta colour developed in the filtrate on adding sodium bismuthate was matched against a standard after centrifuging. Iron was estimated by the micromethod described by Straub [1934]. pH was estimated colorimetrically with the 'Hellige comparator' and standard colour discs.

### EXPERIMENT 1

The data from this experiment are given in Table I. The experiment was continued for three weeks starting with one-week old seedlings.

TABLE I

Uptake of nitrogen by  $NH_4$ - and  $NO_3$ -plants in diffuse light

	NH <sub>4</sub> series	$\mathrm{NO_3}$ series
Dry weight per 32 plants — shoot . " " " " total . Total uptake of N during the experiment	568·3 mg. 122·8 mg. 691·1 mg. 20·14 mg.	571·6 mg. 194·8 mg. 766·4 mg. 17·18 mg.

It will be seen that the NO<sub>3</sub>-plants made as good growth as the NH<sub>4</sub>-plants, the root having made a decidedly better growth in the former. Seedlings (at the fourth leaf stage) from each culture were subsequently set up in one-litre beakers with a view to observing their further growth. Culture solutions of the same strength and composition were used and were renewed at ten day intervals. At the end of the fortnight the first three leaves in the NH<sub>4</sub>-set had withered and before the end of another fortnight all the plants were dead. After three months flowering spikes appeared in the NO<sub>3</sub>-plants. There was no development of tillers in these plants.

## EXPERIMENT 2

In order to see whether FePO<sub>4</sub> was a suitable source of iron for nitrate cultures another experiment was carried out with the same nitrate culture solution as used in Experiment 1 but with FeSO<sub>4</sub> and FePO<sub>4</sub> as sources of iron. FePO<sub>4</sub> was prepared according to Livingstone's procedure as described by Gines [1930]. The experiment was continued for 20 days. Chlorosis developed in the FePO<sub>4</sub> series after about twelve days, thus indicating that the iron from FePO<sub>4</sub>, which was in colloidal form, was not readily available to the plants. Similar results have been recorded by Jones and Shive [1921]. The total dry weight produced and the total uptake of nitrogen were both less in the FePO<sub>4</sub> treatment.

It will thus be seen that even in diffuse light it is possible to grow rice up to the flowering stage, provided care is taken to supply iron in a suitable form. That rice does not thrive in NH<sub>4</sub> cultures during later stages is also evident. In view of the above finding it is surprizing that Macasaet [1936] working in Espine's laboratory, should state: 'But no culture has yet been found that could bring the rice plant to complete maturity. Espino [1920] and Espino and Estioko [1931] succeeded in growing rice plant normally for the first thirty or forty days in certain culture solutions. Beyond that stage of development, however, the plant always produced chlorotic young leaves.'

It may also be noted that Gericke [1930] succeeded in growing plants to maturity in NO<sub>3</sub> cultures with ferric tartarate as the source of iron.

It will be noted from Experiment 1 that the absorption rates of both NO3 and NH4 nitrogen are remarkably low. Such low absorption rates have also been obtained by Dastur and Malkani [1933]. The slow growth rate of the plants under low light intensity (diffuse light) would seem to account for these low absorption rates. In order to see what kind of growth curve obtained under these conditions, during early stages, an experiment was carried out in which dry weight data were collected every two days. Seedlings about a week old were used to begin with (4/10/37) and the nitrate culture was used. In Fig. 1 is shown the curve of increase in dry weight in mg. per five plants (mean of 2 replicates). Mean temperature (mean of 2 maximum and 2 minimum temperatures) during each interval is also shown in Fig. 1.

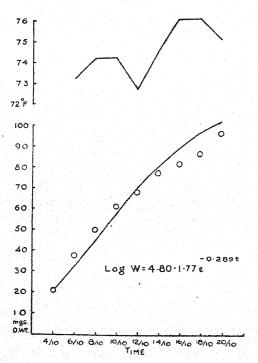


Fig. 1. Increase in dry weight in diffuse light

It will be seen that the growth curve is of a decrement type in which the growth rate is continuously falling with time. Similar growth curves have also been obtained by Gregory [1928] for the surface growth of cotyledons and leaves of cucumber in artificial light at supra-optimal temperatures and by White [1936] for the growth of *Lemna* under potassium deficiency in the culture medium. White

[1936] has fitted to this type of growth curve an equation of the type,  $\frac{1}{n} \cdot \frac{dn}{dt} = re^{-kt}$ , which assumes that the growth rate itself is falling exponentially with time due to an inhibiting factor, which in our case would seem to be low light intensity. The smooth curve shown in Fig. 1 is based on the equation  $\log \frac{n}{no} = \frac{r}{k}$  (1-e<sup>-kt</sup>) and it will be seen that the agreement is moderately close. This observation might account for the very small amount of growth observed by Dastur and John [1938] during the second fortnight from the time of sowing during which they carried out their experiment and for the low rate of nitrogen uptake as reported by Dastur and Malkani [1933]. Although neither Dastur and John [1938] nor Dastur and Malkani [1933] mention what light conditions prevailed during their experiments, it would seem, judging from their absorption rates and growth data, that their experiments were carried out under sub-optimal light conditions.

## EXPERIMENTS IN SUNLIGHT

With a view to studying the growth and nitrogen uptake rates obtaining at a higher light intensity an experiment was carried out in the cage, exposed to sunlight as already described.

### EXPERIMENT 3

In this experiment both growth rates and uptake of nitrogen were measured. Weather throughout the experimental period was very clear and the experiment was continued for about three weeks. Both dry weights and mean temperature are plotted in Fig. 2. The curve of dry weight increase, as given in Fig. 2, conforms very well with that of an exponential type and agreement with the smooth curve is very good considering that no attempt was made to control the environment. The curve of daily uptake of nitrogen approximately followed the dry weight curve, shown in Fig. 2, and the total uptake in 18 days was 191-08 mg. per 25 plants.

With a view to observing further growth in sunlight a few plants were transferred to one litre beakers with the same culture solution renewed periodically. These plants tillered well and flowered after about

14 weeks.

With a view to elucidating the interrelations between Fe, NH<sub>4</sub> and NO<sub>3</sub> ions experiments on a more elaborate scale were carried out. Tall beakers, one litre in capacity carrying seven plants each, were used. Culture solutions of higher concentration were used so that they would be required to be renewed once a week only. The composition of the

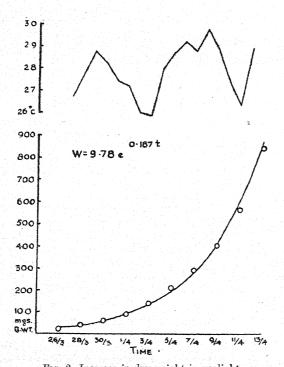


Fig. 2. Increase in dry weight in sunlight three culture solutions used from time to time is given below:—

NO <sub>3</sub> culture	NH4 culture	NH4 + NO3 culture	
Ca (NO <sub>3</sub> ) <sub>2</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	Ca(NO <sub>3</sub> ) <sub>2</sub> +(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	
K <sub>2</sub> SO₄ KH <sub>2</sub> PO₄ Mg SO₄	$egin{array}{c} K_2SO_4 \ KH_2PO_4 \ Mg\ SO_4 \ Ca\ SO_4 \end{array}$	K <sub>2</sub> SO <sub>4</sub> KH <sub>2</sub> PO <sub>4</sub> Mg SO <sub>4</sub> Ca SO <sub>4</sub>	

Boron and manganese were always added to each culture solution in concentration of 1 p.p.m.

#### EXPERIMENT 4

Seeds soaked on 20/4/38. Seedlings were placed in apparatus on 26/4/38. Concentrations of culture solutions as used from time to time were as below:

Date	Mg	Ca	P <sub>2</sub> O <sub>5</sub>	KgO	N	Fe
26/4-3/5 .	24	60	12:3	25.0	42	8 p.p.m.
3/5-10/5 .	24	60	12.3	25.0	42	8 "
10/5-13/5	24	60	12.3	25.0	42	8 "
13/5-16/5 .	24	60	12:3	25.0	42	8 "
16/5-23/5	24	180	24.6	67.0	126(NO <sub>3</sub> )	8 .,,

 $m NH_4$  cultures contained 84 p.p.m. N from 16/5 to 23/5 and  $m NO_3 + NH_4$  cultures had  $m NO_3 = 84$  p.p.m. and  $m NH_4 = 42$  p.p.m.

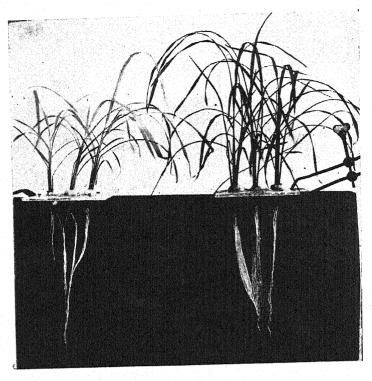
In all six treatments were tried:  $NO_3$ +iron,  $NO_3$ —iron,  $NH_4$ —iron,  $NH_4$ +iron,  $NH_4$ + iron,  $NH_4$ + NO<sub>3</sub>+iron and  $NH_4$ +NO<sub>3</sub>—iron. For the first week all treatments were given iron (8 p.p.m.) as ferric tartarate. From 3/5/38 onwards the minus iron treatments were not given any ferric tartarate. Although the cultures were not aerated there could not have been a serious shortage of oxygen as these had to be replenished with water twice a day due to its loss through transpiration.

The mean number of tillers per plant at the end of the experiment was as follows:

$ m NO_3 + Fe$	NO <sub>3</sub> —Fe	NH <sub>4</sub> +NO <sub>3</sub> +Fe	$\mathrm{NH_4} + \mathrm{NO_3} - \mathrm{Fe}$	$ m NH_4{+}Fe$	NH <sub>4</sub> —Fe
2·75±0·13	1·08±0·08	2·60±0·16	3·25±0·13	1·50±0·15	2·25±0·18

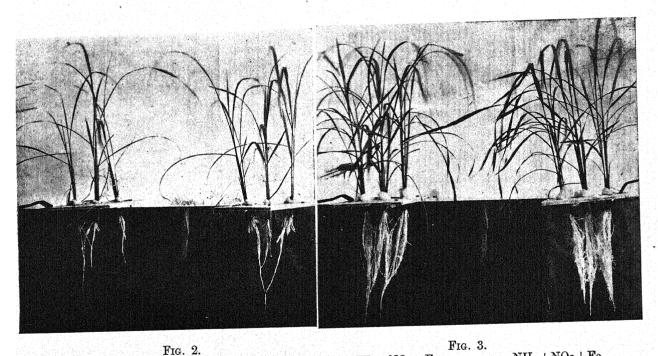
Senescence set in very rapidly in NH<sub>4</sub> plants starting from the leaf tips. The root system in the NO<sub>3</sub> plants was long, white and healthy. In about a week after starting the experiment it was noticed that the secondary roots of both NH<sub>4</sub> and NH<sub>4</sub> + NO<sub>3</sub> plants ceased to grow in length. Later on a thick bunch of root initials surrounded the base of the

shoot in the NH<sub>4</sub> plants which did not elongate. The NH<sub>4</sub>+NO<sub>3</sub> plants had a thick bunch of white adventitious roots which were quite healthy, while those of NH<sub>4</sub> plants were brown and unhealthy. Although the dry weights were the same the character of the root system in NH<sub>4</sub>+NO<sub>3</sub> plants was strikingly different (Plate VIII).



NO<sub>3</sub>—Fe (pH 6·0)

Fig. 1.  $NO_3+Fe~(pH~6.0)$ 



NH<sub>4</sub>—Fe (pH 4·7)

Fig. 2. NH<sub>4</sub>+Fe (pH 4·7)

NH<sub>4</sub>+NO<sub>3</sub>—Fe (*p*H 4·7)

NH<sub>4</sub>+NO<sub>3</sub>+Fe (pH 4·7)



Table II

Mean dry weights in gm. (d.w.) and water content as percentage of dry weight (w.c.) per sample of 6 plants (mean of two replicates) on 23/5/38

		Leaf	Sheath	Shoot	Root	Total
						Name and Address of the Address of t
$\begin{array}{cccccccccccccccccccccccccccccccccccc$		$\begin{array}{c} 2.9631\pm\\ 0.1124\\ 228\\ 2.9286\pm\\ 0.2244\\ 226\\ 2.6889\pm\\ 0.0179\\ 233\\ 1.3664\pm\\ 0.1103\\ 273\\ 1.3580\pm\\ 0.033\\ 185\\ 1.3641\pm\\ 0.099\\ \end{array}$	$\begin{array}{c} 2 \cdot 0979 \pm \\ 0 \cdot 0199 \\ 487 \\ 2 \cdot 0114 \pm \\ 0 \cdot 1687 \\ 510 \\ 1 \cdot 8114 \pm \\ 0 \cdot 0348 \\ 544 \\ 0 \cdot 6654 \pm \\ 0 \cdot 0595 \\ 750 \\ 0 \cdot 9918 \pm \\ 0 \cdot 0164 \\ 460 \\ 1 \cdot 1561 \pm \\ 0 \cdot 1023 \\ \end{array}$	5·0612 ··· 4·9400 ··· 4·5003 ··· 2·0318 ··· 2·3498 0·019 ··· 2·5202	$\begin{array}{c} 0.9231\pm\\ 0.0547\\ 787\\ 0.9122\pm\\ 0.0673\\ 786\\ 0.8725\pm\\ 0.0263\\ 749\\ 0.3990\pm\\ 0.0239\\ 751\\ 0.2823\pm\\ 0.0219\\ 625\\ 0.1961\pm\\ 0.0034\\ \end{array}$	$5.9843 \pm 0.1472$
w.c.		181	375	••	626	

Although the dry weights of each plant part of both NH<sub>4</sub>+NO<sub>3</sub> series is higher than that of NO<sub>3</sub> + Fe, the difference does not attain the level of statistical significance which, however, might be due to the small number of replicates. Only NO<sub>3</sub> plants are markedly influenced by iron deficiency. The NH<sub>4</sub> plants have a lower water content. The rates of nitrogen uptake differ approximately in the same direction as the dry weights in different treatments. It may also be noted that the rates of uptake of NO<sub>3</sub> and NH<sub>4</sub> ions are almost the same in

the early stages, which is quite contrary to the observations of others. It must be remembered that the differences during the last week (16/5/-23/5) are due to the differences in the original concentrations of the respective ions.

The iron contents in mg. per gm. dry weight of leaf are given in Table IV. It will be seen that the iron content of NO<sub>3</sub>—Fe and NH<sub>4</sub>—Fe plants is approximately the same in spite of which the leaves in the former series (except the first four leaves) were chlorotic, while those in the latter remained

Table III

Mean uptake of nitrogen in mg. per seven plants (mean of two replicates)

Date	$\mathrm{NO_3} + \mathrm{Fe}$	NO <sub>3</sub> — Fe	$NH_4 + NO_3 + Fe$	$NH_4 + NO_3 - Fe$	NH <sub>4</sub> + Fe	NH <sub>4</sub> — Fe
26/4-3/5	9·11 32·74 28·70 40·71 116·75 228·01	9.26 27.90 16.08 19.50 49.10 121.84	$\begin{array}{c} 6.84 + 6.83 \cdot \\ 20.35 + 20.78 \\ 18.51 + 17.07 \\ 20.20 + 20.08 \\ 44.42 + 72.30 \\ 247.38 \end{array}$	$\begin{array}{c} 6.55 + 5.55 \\ 20.07 + 20.21 \\ 16.37 + 18.36 \\ 20.63 + 19.79 \\ 43.99 + 77.61 \\ 249.13 \end{array}$	8·71 21·53 20·01 20·5 51·67 122·42	10-25 24-43 23-42 28-7 71-74 158-54

Table IV

The iron content in mg. per gm. dry weight of leaf

$ m NO_3 + Fe$	NO <sub>3</sub> — Fe	$NH_4 + NO_3 + Fe$	$NH_4 + NO_3 - Fe$	$NH_4 + Fe$	$\mathrm{NH_4}$ — Fe
0.34	0.1893	0.4675	0.3532	0.4179	0.1772

green. The iron content of  $\mathrm{NH_4} + \mathrm{NO_3} - \mathrm{Fe}$  plants which was as high as that of  $\mathrm{NO_3} + \mathrm{Fe}$  plants might perhaps be accounted for by the lower  $p\mathrm{H}$  of the culture solution which might have dissolved more iron during the first week and subsequently from the impurities of the salts. It might be argued that the  $\mathrm{NH_4} + \mathrm{NO_3}$  plants did not really suffer from iron deficiency and therefore the question whether these plants can really do with smaller amount of iron still remains open.

#### EXPERIMENT 5

Seeds were soaked for about six hours on 8/6/38 and seedlings were placed in apparatus on 15/6/38. Treatments were as under, replicated three times:

(1)  $NO_3$ +Fe (pH 4·7); (2)  $NO_3$ +Fe (pH 6·0); (3)  $NO_3$ -Fe (pH 6·0); (4)  $NO_3$ -Fe (pH 4·7). pH 4·7 was maintained by adding required amount of  $H_2SO_4$ .

Strengths of the culture solutions are given below: 15/6—22/6. Same as in Experiment 4 for the first week.

22/6—29/6. Same as in Experiment 4 for the first week.

 $\begin{array}{l} 29/6-6/7.\ N=84\ ;\ K_2O=42\ ;\ P_2O_5=12\cdot 3\ ;\\ Ca=120\ ;\ Mg=48\ ;\ Fe=8\ p.p.m.\\ 6/7-13/7.\ N=105\ ;\ K_2O=44\cdot 0\ ;\ P_2O_5=15\cdot 0\ ;\\ Ca=150\ p.p.m. \end{array}$ 

Other elements were as used during the first two weeks. Iron as ferric tartarate, was given to all the four series for the first week, after which it was omitted from the deficient series. After about 12 days, the leaves of NO<sub>3</sub>—Fe (pH 4·7) set folded inwards along their midribs and plants from two replicate beakers died off. Plants in the third replicate recovered and afterwards they grew fairly well, but on account of this early setback they cannot strictly be compared with the other treatments. The noteworthy features of these plants were that they did not become chlorotic, while the leaves of the NO<sub>3</sub>—Fe (pH 6·0) plants developed chlorosis from the third leaf onwards. The root system in both NO<sub>3</sub>+Fe (pH 4·7) and NO<sub>3</sub>-Fe (pH 4.7) plants lagged behind those in the other treatments in development during the first week but later many adventitious roots grew out and presented the same appearance as the roots of the NH<sub>4</sub>+NO<sub>3</sub> plants of Experiment 4.

Table V

Mean dry weight in gm. per sample of six plants on 13/7/38

(Mean of three replicates)

NO <sub>3</sub> +Fe (pH 4·7)	NO <sub>2</sub> + Fe (pH 6.0)	NO <sub>3</sub> —Fe (pH 6·0)	NO <sub>3</sub> —Fe (pH 4·7)
MO <sub>3</sub> +re (pit 47)			
$2 \cdot 7674 \pm 0 \cdot 1370$	$3.6756 \pm 0.0572$	$1.8973 \pm 0.1430$	1.8202

The difference due to pH in the  $NO_3$ +Fe treatments is significant. It is not clear whether lower pH or greater uptake of iron is responsible for this depressing effect.

The rates of uptake of nitrogen show the same differences as the dry weights. As from the second week onwards the minus iron plants at  $p{\rm H}$  4·7 began

to dry up and subsequently died, the rate of uptake was very slow in the second week. If we compare, however, the rates during the first week when the treatments were identical, except the differences in  $p\mathrm{H}$ , we see that  $p\mathrm{H}$  had little influence on the rate of uptake of nitrate nitrogen. At  $p\mathrm{H}$  4.7 it was 14.54 and at  $p\mathrm{H}$  6.0, 14.96.

Table VI

Mean rate of uptake of nitrogen in mg. by seven plants

	$NO_3 + Fe (pH 4.7)$ $NO_3 + Fe (pH 6.0)$		NO <sub>3</sub> —Fe ( <i>p</i> H 6·0)	NO <sub>3</sub> —Fe ( <i>p</i> H 4·7)	
15/6—22/6 · · · 22/6—29/6 · · · 29/6—6/7 · · · · 6/7—13/7 · · · ·	5·47 17·70 42·39 54·71	8·20 23·17 51·39 69·11	6·76 16·41 29·80 30·64	9·07 4·03 not estimated do.	
Total •	120-27	151.87	83.64		

Table VII

Iron content in mg. per gm. dry weight

	NO <sub>3</sub> +Fe (pH 4·7)	NO <sub>3</sub> +Fe (pH 6·0)	NO <sub>3</sub> —Fe (pH 6·0)	NO <sub>3</sub> —Fe (pH 4·7)
Leaf Sheath	0·706	0·407	0·340	0·285
	0·493	0·248	0·314	0·280

In this experiment the iron content is much higher in both NO<sub>3</sub>+Fe (pH 6·0) and NO<sub>3</sub>-Fe (pH 6·0) plants as compared to the identical series of Experiment 4. It is probable that this very high content is due to much less growth made in this experiment. Although the leaves in the NO<sub>3</sub>+Fe (pH 4·7) plants were a shade deeper green their efficiency in dry matter production was not evidently increased.

## Experiment 6

Seeds were soaked on 13/7/38 and seedlings set up in apparatus on 20/7/38. Treatments were as under, each having been replicated thrice:

NO<sub>3</sub>+Fe (pH 6·0); NO<sub>3</sub>—Fe (pH 4·7); NO<sub>3</sub>—Fe (pH 6·0); NH<sub>4</sub>+Fe (pH 4·7); NH<sub>4</sub>—Fe (pH 6·0). During the first week all treatments were given iron as ferric tartarate and also all were maintained at pH 4·7 except NO<sub>3</sub>+Fe; from the second week onwards iron was omitted from the deficiency series and the pH values shown in brackets were maintained by daily checking and adding requisite amounts of sulphuric acid or caustic soda. The idea in keeping the pH at 4·7 in all treatments except NO<sub>3</sub>+Fe during the first week was this: at pH 6·0 and higher than this iron tends to precipitate gradually and the roots are covered with a brown insoluble deposit of iron oxides, thus making

the chemical analysis of roots for iron impossible. The other object was to keep the concentration of iron in the two NO3-Fe cultures identical during the first week so that the plants can have the same total iron content. It must, however, be recorded that the shift in pH during the period of 24 hours was quite large, that of NH<sub>4</sub>—Fe (pH 6·0) decreasing by about 1·5 units and that of NO<sub>3</sub>—Fe (pH 4·7) increasing by about 1.3 units. In spite of this incomplete control of pH, the differences obtained were sufficiently striking, although experiments, under better controlled conditions, would certainly seem to be more desirable. Out of three replicates under each treatment one was reserved for the checking of pH. The other two were used for the estimation of the uptake of nitrogen. Although the replicate used for the estimation of pH was deprived daily of its culture solution by 10 to 20 c.c., the deficit thus caused could not be very appreciable because the concentration of each element was sufficiently large. The concentrations of the culture solutions used were as under:

## TABLE VIII

Mean dry weight in gm. (d.w.) per six plants (mean of three replicates) and water content as percentage of dry weight (w.c.) on 17/8/38. Standard errors in NO<sub>3</sub>—Fe (pH 4·7) and NO<sub>3</sub>—Fe (pH 6·0) could not be estimated because the first three and the remaining leaves were weighed separately. As the sample for the first three leaves was very small the three replicates were weighed together.

	Leaf	Sheath	Shoot	Root	Total
NO <sub>3</sub> +Fe (pH 6·0) d.w	1·9874± 0·109 251	$1.5786 \pm 0.1711 \\ 522$	3-5660±	$0.9224 \pm 0.0791 \\ 796$	4·4884± 0·2592
NO <sub>3</sub> —Fe (pH 4·7) d.w. w.e	1·7824 265	1·4298 575	3+2122	0·9281 942	4:1403
NO <sub>3</sub> —Fe (pH 6·0) d,w, w.c.	$0.7571 \\ 264 \\ 1.2519+$	$0.4587 \ 652 \ 1.1452 \pm$	1·2098 2·3971	$0.2864 \\ 960 \\ 0.2249 +$	1,4962 2,6220+
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$egin{array}{c} 10.2319 \pm \\ 0.04 \\ 176 \\ 1.5829 \pm \\ \end{array}$	$ \begin{array}{c c} 0.1576 \\ 354 \\ 0.9508 + \end{array} $	2.5338 .	$ \begin{array}{c} 0.01403 \\ 631 \\ 0.4069 + \end{array} $	0.2108 2.9407±
d.w	0·2444 257	0·2095 647		0·0346	0.4784

The marked effect of higher pH is indicated by the increased dry weight of the root system of  $NH_4$ —Fe (pH 6·0) plants. One might, therefore, conclude with some justification that it is the low pH that inhibits root development rather than  $NH_4$  ion per se. Not only has the dry weight increased but also the water content in every part of the plant. The individual leaves also died off more slowly than those of the plants grown at pH 4·7. Another remarkable feature was that no chlorosis developed in the leaves in spite of deficiency of iron, while it did in the case of  $NO_3$ —Fe (pH 6·0) plants (from the fourth leaf onwards). Chlorosis did not develop in the  $NO_3$ —Fe (pH 4·7) plants and they made almost as good growth as the  $NO_3$ +Fe (pH 6·0) plants. The  $NO_3$ —Fe (pH 4·7) plants were also characterized by a short root system with a thick

bunch of short adventitious roots of the type that developed in the NO<sub>3</sub>+NH<sub>4</sub> plants of Experiment 4 and NO<sub>3</sub>+Fe (pH 4·7) plants of Experiment 5. Evidently there was better utilization of iron in this case.

The rates of uptake of nitrogen followed the same trend as the dry weights. As has already been pointed out, during the first week all the treatments, except  $NO_3$ +Fe, were maintained at pH 4·7. It will be seen that at pH 4·7 there was no greater uptake of nitrate nitrogen than at pH 6·0. Similarly during the first three weeks there was little difference in the uptake of ammonium at pH 4·7 and 6·0 respectively. Later on the plants at pH 6·0 developed better root system and also their leaves did not age as quickly and hence we find a greater uptake of ammonium.

Table IX

Mean rate of uptake of nitrogen in mg. per seven plants

	NO <sub>3</sub> +Fe (pH 6.0)	NO <sub>3</sub> —Fe (pH 6·0)	NO <sub>3</sub> —Fe (pH 4·7)	$ \begin{array}{c} \mathrm{NH_4} + \mathrm{Fe} \\ (p\mathrm{H} 4.7) \end{array} $	NH <sub>4</sub> —Fe (pH 6·0)
20/727/7 · · · · · · · · · · · · · · · · · ·	7·05 22·74 63·33 119·62	6·16 14·10 33·40 37·13	7·05 17·85 56·43 114·87	5·90 21·42 48·53 52·51	7·46 16·58 48·87 79·11
Total .	212.74	90.79	196-20	128.38	152.02

Table X

Iron content in mg. per gm. dry weight

Treatment	Leaf	Sheath	Root
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	0·465 0·262 0·395 0·456 (chlorotic) 0·427 (green) 0·329 (upper) 0·589 (lower 3)	0·316 0·250 0·383 0·187 0·456 0·273 0·694	1:88 0:701

In the NO<sub>3</sub>—Fe (pH 6) leaves the iron content was higher than that of the NO<sub>3</sub>+Fe ones. Although the iron content of NO<sub>3</sub>—Fe (pH 4·7) leaves was lower than that of the NO<sub>3</sub>—Fe (pH 6) ones there was no chlorosis in the former. Although the NH<sub>4</sub>—Fe (pH 6) leaves contained much less iron than the NH<sub>4</sub>+Fe ones they did not develop chlorosis.

The calcium content of NH<sub>4</sub>-leaves was markedly less than that of the NO<sub>3</sub> ones. At lower pH the Ca content was much less in both NO<sub>3</sub> and NH<sub>4</sub>-leaves.

Striking differences in the manganese\* content of the plants of different treatments were also

<sup>\*</sup> Manganese could not be estimated quantitatively for want of time. The differences were judged only qualitatively,

found. The NH<sub>4</sub>-plants were characterized by the lowest content of manganese. The highest content was found in the first three green leaves of the NO<sub>3</sub>—Fe (pH 6) plants, followed by the chlorotic leaves of the same.

Table XI

Calcium (as CaO) content as percentage of dry weight of leaves

Treatme	nt		Leaf	
part (1875) (1875) (1886) (1886) (1886) (1886) (1886) (1886) (1886) (1886) (1886) (1886) (1886) (1886) (1886)			et en termina in communication de la communica	
XH <sub>4</sub> - Fe (pH 4-7)		-	0.428	
NH <sub>4</sub> —Fe (pH 6-0)			0.710	
$NO_a + Fe \left( pH(6.0) \right)$			1.512	
$\mathrm{NO}_{3}$ —Fe ( $p\mathrm{H}(4.7)$	•		1-125	

#### Discussion

## A. Influence of light and temperature

We have already noted the differences in the type of growth curves obtained in diffuse and sunlight respectively (Experiments 3 and 4). We may now briefly refer to the influence of this factor as well as temperature on the other experiments for which the relevent data are given in Table XII.

Table XII
Influence of light and temperature on experiments

Experi- ment	Treatment	Mean dry weight	Mean hours bright sunshine per day	Mean temperature
<b>4</b> 5 6	$\begin{array}{c} NO_3 + Fe \; (pH \; 6) \\ NO_3 - Fe \; (pH \; 6) \\ NI_4 + Fe \; (pH \; 6) \\ NO_3 + Fe \; (pH \; 6) \\ NO_3 - Fe \; (pH \; 6) \\ NO_3 + Fe \; (pH \; 6) \\ NO_3 - Fe \; (pH \; 6) \\ NO_4 - Fe \; (pH \; 6) \\ NH_4 + Fe \; (pH \; 4 \cdot 7) \end{array}$	5-3729 gm. 2-4308 2-6321 3-6756 1-8973 4-4884 1-4962 2-6220	8-9 8-9 8-9 3-9 3-9 4-9 4-9	86·8°F. 86·8°F. 86·8°F. 78·4°F. 78·4°F. 78·7°F. 78·7°F.

The influence of both light and temperature is evident in the case of NO<sub>3</sub>+Fe and NO<sub>3</sub>-Fe treatments. The reason why the NO<sub>3</sub>-Fe plants of Experiment 6 produced less dry weight than those of Experiment 5, in spite of one more hour of bright sunshine per day, may be that the mean hours of bright sunshine during the first two weeks were 2.6 and 4.4 hours respectively as against 3.9 and 6.9 hours during Experiment 5. Later, although the light conditions improved the plants could not possibly make up because of iron deficiency. The NH<sub>4</sub>-plants, on the other hand, do not show any influence of light and temperature and it is not possible to account for this result in the absence of more data.

As we have already seen that the dry weights and the total rates of nitrogen uptake vary in the same direction, it would be interesting to compare the weekly rates of nitrogen uptake with the weekly means of hours of bright sunshine per day (Table XIII).

It will be seen that there is a close correlation in the case of NO<sub>3</sub>+Fe and NO<sub>3</sub>-Fe treatments only. It is not suggested that light intensity influences the absorption process. The latter is fluenced through the acceleration of the growth rate by a higher light intensity.

## B. Iron chlorosis

The percentage iron content of chlorotic leaves has been found to be nearly the same as or lower than that of green leaves and it is generally held that iron exists in an insoluble and therefore unavailable form in chlorotic leaves. Indeed, sap analyses have invariably shown that green leaves contain more soluble iron than chlorotic leaves. Olsen [1935] found very high total phosphorus

Table XIII

Comparison of weekly rates of nitrogen uptake and weekly means of hours of bright sunshine per day

	lst week	2nd week	3rd week	4th week
Hours of bright sunshine . NO <sub>3</sub> +Fe (Experiment 4) . NO <sub>3</sub> +Fe (Experiment 4) . NO <sub>3</sub> +Fe	. 8-6	9·3	8·8	9·0
	. 9-11	32·74	69·41	116·75
	. 9-26	27·90	35·58	49·10
	. 8-71	21·53	40·51	51·67
	. 2-6	4·4	7·0	5·9
	. 7-05	22·74	63·33	119·62
	. 6-16	14·10	33·40	37·13
	. 5-90	21·42	48·53	52·51
	. 3-9	6·9	3·2	1·6
	. 8-2	23·17	51·39	69·11
	. 6-76	16·41	29·80	30·64

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content associated with chlorotic leaves and he held that iron was, therefore, precipitated as ferric phosphate. Since phosphorus exists in the leaves in several forms and combinations, Olsen's suggestion would seem to be far too simple. Wadleigh, Robbins and Beckenbach [1937] believed that their observation of an association between high soluble phosphorus content (in sap) and chlorosis accorded with Olsen's suggestion; this, however, is not evidently true. Chapman [1931] suggested that manganese by stimulating oxidase action converts iron into an insoluble ferric form. This does not seem to account for chlorosis observed in the present work as manganese was found to be in much higher concentration in the first three green leaves of chlorotic plants. Ingalls and Shive [1931] observed that there was a direct relationship between hydrogen ion concentration and soluble iron content in tissue fluids. Chapman [1931], Oserkowsky [1932] and Wadleigh, Robbins and

Beckenbach [1937], however, did not find any such relation in chlorotic leaves. With a view to testing this point the pH of sap of plants of Experiment 6 was estimated by means of 'Wulff pH tester'. In a few drops of the extracted sap a small strip of indicator foil was dipped for about a minute and the colour developed in the strip matched with standard colour strips. The results are given in Table XIV. It will be seen that there is no association between chlorosis and pH. It appears that although other workers have found a much higher base content associated with chlorosis, they have not regarded this as of any significance. From an inspection of the data of Wadleigh and Shive [1939] and Olsen [1935] on corn grown in nitrate culture it appears that the total base content and soluble iron content are negatively correlated. The percentage ash content of different plant tissues from Experiment 6 also show the same trend (Table XV).

Table XIV

pH of sap of plants of Experiment 6

pH of culture solution	Treatment	Tissue	pH of sap	
	$\mathrm{NO_3}\mathrm{+Fe}$	Leaf	6.2	
·0	NH <sub>4</sub> +Fe		5.6	
	NO <sub>3</sub> —Fe		6.0	
0	NH <sub>4</sub> —Fe		6.2	
.0		Root	6.2	
•0	NO <sub>3</sub> —Fe		6.2	
•0		Leaf	6.2	

Table XV

Percentage ash content of different plant tissues from Experiment 6

Treatment	pH	pH Leaf		Root	
NH <sub>4</sub> +Fe ,	. 4.7	6.6	4.0		
NH <sub>4</sub> —Fe	. 6.0	8:7	9.2		
NO <sub>3</sub> +Fe · · · · · · · · · · · · · · · · · · ·	. 6.0	6:4	6.6		
NO <sub>3</sub> —Fe · · · · · · ·	6:0	12·9 (chlorotic)	14.0	14:7	
를 보냈다는 마리를 위하는 것 같습니다. 같은 모든 다음을 받았다고 하였다. 보통하다 기계 전략 기계 하다. 경우 기계를 보냈다. 하는 것은 사람들은 말라	6.0	11-2 (green)	5.1		
	4.7	6.5	7.9	5.7	
,,	. 4.7	4.0 (first three)	1.0		

The total uptake of ash by NO<sub>3</sub>—Fe (pH 6.0) plants amounted to 190.6 mg. per six plants and by NO<sub>3</sub>-Fe (pH 4.7) plants to 270.6 mg. whereas the total dry weights were 1.49 and 4.14 gm. respectively. In Table XVI is shown the distribution of iron in plants from Experiment 6. At pH 4.7 distinctly higher amount of iron has been absorbed and this might have been supplied from impurities in the culture solution remaining dissolved at the lower pH. It may be noted that 55 per cent of the total iron content was retained in the roots of plants at pH 6.0 as against 38 per cent at pH 4.7. The differences in the sheath are similarly wide. It appears as if iron is not transferred as rapidly from roots at pH 6.0 as from those at pH 4.7. It is worth recording that subsequent to the change in pH from 4.7 to 6.0 after the first week of the experiment the roots elongated considerably more within 48 hours. How this root behaviour is associated with

the retention of a larger proportion of iron in the root is difficult to visualize. Is it possible that the relatively larger absorption of other ash constituents at pH 6.0 interfered with the transport of iron? The data presented in Table XVII bring out this point more clearly. It will be seen that the water content per unit quantity of ash is considerably less in NO<sub>3</sub>—Fe (pH 6.0) leaves. The question may well be raised as to why greater uptake of ash did not take place in the NO3+Fe-plants maintained throughtout at pH 6.0. With the data at hand it is difficult to answer this question. It can only be surmised that pH 6.0 per se cannot have stimulated the uptake of ash by NO3-Feplants. The question whether better utilization of iron could occur at the lower pH is also not sufficiently answered by the data at hand. Experiments with highly purified salts and direct estimation of iron in such a culture medium can settle this point.

Table XVI

Distribution of iron in plants from Experiment 6

NO <sub>3</sub> —Fe ( <i>)</i>	оН 6.0)		NO <sub>3</sub> —Fe (pH 4·7)				
	Absolute iron content in mg.	Percentage of total		Absolute iron content in mg.	Percentage of total		
First three green leaves	0·07122 0·26643 0·06674 0·04641 0·53845	4·3 29·9 6·8 4·0 55·0	First three green leaves . Remaining green leaves . Sheath (remaining leaves) . Sheath (first three green leaves) . Root	0.09200 0.53500 0.36127 0.07384 0.65061	5·0 32·0 21·0 4·0 38·0		
	0.98925	100-0		1.71272	100-0		

Table XVII

Water, iron and ash contents of leaf

	NO <sub>8</sub> —Fe (pH 6·0)	NO <sub>3</sub> —Fe (pH 4·7)	NO <sub>3</sub> +Fe (pH 6·0)
Water content in mg. per unit leaf area in sq. in.	11.67	16.09	17.70
Tron content in γ* per unit leaf area in sq. in.	1.85	1.94	2.70
Ash in mg. per unit leaf area in sq. in.	0.52	0.38	0.45
Water content in mg. per mg. ash	22-20	42.00	39.30

Y\* (gamma) is equal to 0.001 mg.

The data on the first three green leaves of the  $NO_3$ —Fe (pH 6.0) and on the corresponding ones of the pH 4.7 treatments are interesting and are presented in Table XVIII.

TABLE XVIII

Iron, water and ash contents of the first three green leaves of  $NO_3$ —Fe  $(pH \ 6.0)$  and  $(pH \ 4.7)$ 

	NO <sub>3</sub> —Fe (pH 6.0)	NO <sub>a</sub> —Fe (pH 4·7)
Iron content in y per unit area (sq. in.)	10.66	13.78
Water content in mg. per unit area ( ,, )	41-90	42.26
Ash content in mg. per unit area ( ,, )	2.80	0.986
Dry weight in mg. per unit area ( ,, )	23-20	24.97

Again the difference in ash content is very striking. This higher uptake of ash must have occurred after the first week of the experiment when the pH was raised to 6.0. Presumably iron existed in a soluble and available state during the first week with the

result that there was no interference with the development of chlorophyll.

It has already been pointed out that no chlorosis developed in the leaves in the NH4-Fe cultures (Experiments 4 and 6), although their iron content was nearly 50 per cent less (Tables IV and In Experiment 4 the pH of both NH, cultures was 4.7 and they gave equally good growth. In Experiment 6, however, the plants in the treatment NH .- Fe (pH 6-0) made much better growth than those in the treatment NH<sub>4</sub>+Fe (pH 4.7). Can the plants grown in NH<sub>4</sub> cultures do with a smaller concentration of iron? Wadleigh, Robbins and Beckenbach [1937] observed that maize plants grown in NH, cultures were relatively free from chlorosis as compared to those grown in NO3 cultures, while Gaertner [1937] did not find this to be the case with maize and Hydrangea hortensis and Ross [1938] with maize, Ricinus communis and Lupinus alba. It may be noted, however, that the latter two workers altogether omitted iron from their cultures. Under the cultural conditions adopted by the author the plants in NH, cultures did not make as good growth as those in NO3 cultures and probably the effect of iron deficiency was masked on this account. Thus the question whether NH, plants could do with less amount of iron would appear to remain open. Neish's [1939] observation is interesting in this connection as he finds that copper, iron, phosphorus and ammonium salts are concentrated to a certain degree in the chloroplasts while sulphate and nitrate do not follow any general rule. Is it possible that iron exists in a soluble state within the chloroplast by combining with some ammonium compound?

## C. Growth of rice plant in NH , cultures

It has already been noted that pH had little influence on the rate of uptake of ammonium and that the plants were characterized by an unhealthy root system and rapid senescence of the older leaves. Jacobson and Swanback [1933] found in tabacco grown in a mixture of nitrate and ammonium a form of root rot which increased in severity as the proportion of the latter increased. Low pH (4.7) would seem to be mainly responsible for this as the data of Experiment 6 indicate. At this pH the root growth slowed down in both nitrate and ammonium cultures and later adventitious roots grew out profusely in the former while they just emerged but did not clongate in the latter. Apparently the supply of carbohydrate became limited since it is generally held that ammonium depletes the leaves their carbohydrate considerably of [Nightingale, 1933-34, Sideris et al., 1938]. It is also probable that the balance of uptake of other mineral elements might have been adversely influenced as the calcium content (Table XI) and the ash content (Table XV) indicate and this might

in turn have affected carbohydrate metabolism. Jacobson and Swanback [1933] found that increased proportion of nitrate over ammonium resulted in a larger percentage of calcium in the plant material and Holley, Pickett and Dulin [1931] observed that ammonium definitely reduced calcium absorption by cotton. It is also significant that there was no response to higher light intensity in Experiment 4 as compared to Experiment 6.

#### SUMMARY

Experiments with rice in both diffuse light and sunlight were carried out in complete culture solutions containing ammonium and nitrate nitrogen. In diffuse light (not direct sunlight) the relative growth rate was found to fall continually with time and the uptake of both forms of nitrogen was low, whereas in sunlight the total dry weight increased more or less exponentially and the uptake of nitrogen was high. It is suggested that light intensity might have been a limiting factor in the low nitrogen uptake and the small amount of growth reported by previous workers.

The source of iron appeared to be related to the poor growth and chlorosis resulting in nitrate cultures. With ferrous sulphate the nitrate cultures gave much better growth than with ferric phosphate. With a suitable source of iron it was possible to grow plants in a nitrate culture solution up to the flowering stage even in diffuse light although no tillering occurred. In sunlight the nitrate plants produced a large number of tillers and all of them flowered. It was not possible to grow healthy plants for long in ammonium cultures at low pH.

In an experiment with different complete culture solutions consisting of (1) ammonium, (2) ammonium + iron, (3) nitrate, (4) nitrate + iron, (5) ammonium+nitrate+iron, and (6) ammonium+nitrate respectively, chlorosis developed in (3), root development was restricted in (1) and (2) and the root system was about but profuse in (5) and (6)

was short but profuse in (5) and (6).

In another experiment with complete culture solutions consisting of (1) nitrate at pH 6.0, (2) nitrate+iron at pH 6.0, (3) nitrate at pH 4.7, (4) ammonium at pH 6.0, and (5) ammonium+iron at pH 4.7 respectively, chlorosis developed in (1), the plants grew equally well in (2) and (3) and the plants in (4) developed quite a healthy root system and the leaves did not age rapidly as compared to (5).

The differences in dry weights and rates of nitrogen uptake, obtained in different nitrate cultures at different times, appeared to be correlated with differences in the mean number of hours of bright

sunshine during the experimental period.

It appeared that pH not only profoundly influenced the uptake of mineral elements but also the character of the root system. During comparable growth stages  $p\mathbf{H}$  did not influence the rate of uptake of

either nitrate or ammonium nitrogen.

Chlorotic leaves showed a much greater reduction in dry weight than in area. The iron content, on dry weight basis, therefore, appeared as high or even higher than that of green leaves. On leaf area basis the differences in ash content, water content and iron content were noteworthy and it is suggested that chlorosis was brought about not only by reduction in iron content but also by depression of its solubility by higher ash content and lower water content per unit area.

Although there was some evidence that ammonium plants could thrive on a much smaller concentration of iron in the culture medium, the question

still remained open.

## ACKNOWLEDGEMENTS

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## STUDIES ON THE VIRUS DISEASES OF POTATOES IN INDIA

II. SOLANUM VIRUS 2 (ORTON.)

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(With Plates IX and X)

SOLANUM VIRUS 2 (Potato virus Y, Smith) was recovered during analytical work on potato viruses from potato plants of Phulwa variety showing either negligible mosaic and veinal necroses or severe mosaic only. The virus was also cultured from plants of Gola, Majestic, President, Windsor Castle and Talisman (Up-to-date) varieties showing mosaic and veinal necroses followed by acropetal necrosis. All the varieties excepting certain Phulwa plants showing negligible mosaic and veinal necroses vielded a mixture of Solanum viruses 2 and 1. The composite nature of diseases in these varieties was indicated by specific reactions on certain plant indicators. The separation of Solanum virus 2 from the mixture was effected by passage of the complex through Petunia hybrida.

The symptoms exhibited by potato plants of some of the varieties from which Solamum virus 2 was

isolated are described below.

Phulwa. The virus in this variety was isolated from plants showing two different types of symptoms, i.e. negligible mosaic with veinal necroses and severe type of mosaic. In the former case the growth of plants is seldom stunted. The plants appear normal and it is hard to read the mosaic symptoms on them. Veinal necroses is present on the under surface of the leaflets. Necroses may commence on the midrib or the minor veins and often reach the petiole, though the occurrence of leaf-drop streak has not so far been observed. Leaves affected with veinal necroses show palish streaks along the midrib and the veins on the upper surface indicating corresponding veinal necroses on the under surface. The texture of the leaves is unaltered but the leaf apex and sometimes even the leaf margins tend to point downwards. The general colour of the foliage of the affected plants, except those showing severe symptoms of infection, is not appreciably changed and the disease may, at times, pass off unnoticed failing a careful examination. Plate IX, fig. 1 shows a Phulwa plant leaf with veinal necroses. In the case of severe types of mosaic there is intense

mottling of the leaf surface. Palish or yellowish areas are visible all over the leaf surface except in the centre where a small patch of green may persist. Sometimes the whole leaf may turn yellowish. The plants are stunted and the leaflets are greatly reduced in size, show distortion and puckering, and develop

purple colour. The general appearance of the foliage is extremely pale. Vein bending is frequently observed and may at times be very prominent. The leaflets are wrinkled, thick, at times almost saucer-shaped and birttle. Necrosis is absent.

Gola. Plants are stunted and assume an erect habit. The upper leaves show negligible or mild mottle, reduction in size, thickening and some distortion. Lower leaves are not appreciably reduced in size. The leaflets are thick and brittle and their tips point in a downward direction. Brown or dark brown veinal necroses are observed on the under surface of leaves. In severe cases the necroses may extend to the petiole and result in leaf-drop streak. Later on the necroses may be visible on the upper surface of leaves. Some leaves may even show necrotic spots. Plate IX, fig. 2 shows a plant of Gola variety affected with leaf-drop streak and fig. 3 shows leaflets of the plant with veinal necroses and necrotic spots.

The symptoms of the disease in general in potato varieties Majestic, President, Windsor Castle and Talisman (Up-to-date) resemble those described for

Gola.

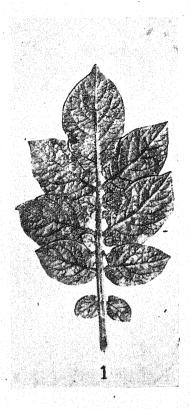
The reactions of the cultures of the virus obtained from different potato varieties were studied on differential hosts and it was observed that the symptoms produced by different cultures on the differentials were almost similar.

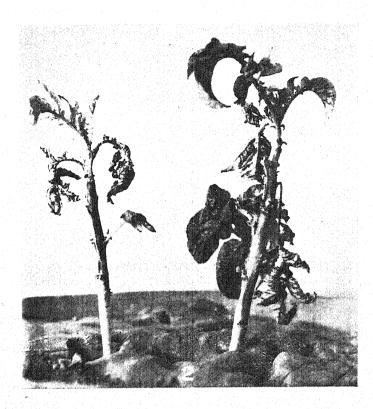
The properties and reactions on differential hosts of the virus originally isolated in a pure form from a plant of Phulwa variety showing mosaic and veinal necroses are reported.

#### Reactions on differential hosts

A study of the symptom expression of the virus was carried out on a selected range of solanaceous plants including Nicotiana tabacum L., Nicotiana glutinosa L., Nicotiana sylvestris Spegaz and Comes, and Nicotiana rustica L., Datura stramonium L., Solanum nodiflorum Jack., Petunia hybrida Vilm. and potato variety President. The reactions of the virus on these hosts are described.

Nicotiana tabacum (varieties Harrison's Special and White Burley). Primary symptoms of infection are visible about 15-17 days after inoculation in the form of clearing of the veins of young leaves. A couple of days later, vein clearing becomes very





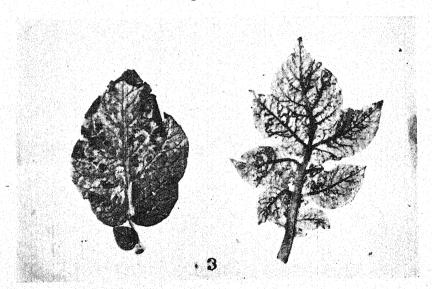
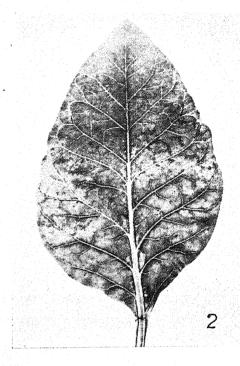
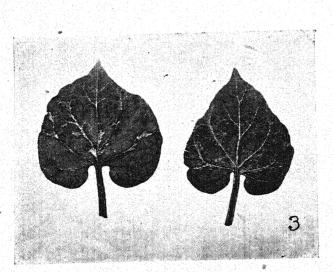
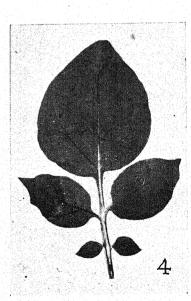


Fig. 1. Leaflets of potato variety Phulwa showing veinal necroses
Fig. 2. Potato plant of variety Gola showing leaf drop streak
Fig. 3. Leaflets of the Gola plant showing veinal necrosis and necrotic spots









Figs. 1 and 2. Leaves of *Nicotiana tabacum* showing vein clearing and green vein-banding Leaves of *Nicotiana glutinosa* showing green vein-banding Leaflets of Potato variety President showing veinal necroses

distinct. After a further period of about four days vein-clearing tends to fade and is followed by a darkening of the green colour in the tissues along the veins. Green vein banding which is usually very prominent is a characteristic symptom of infection by this virus (Plate X, figs. 1 and 2).

by this virus (Plate X, figs. 1 and 2).

Nicotiana glutinosa. Primary symptoms, i.e. clearing of the veins of young leaves, occur in about 15 days after inoculation. A week later vein-clearing disappears and is replaced by green vein-banding. At times leaves also show some distortion (Plate X,

Nicotiana rustica and N. Sylvestris. Clearing of the veins of the young leaves commences about 17 days after inoculation. About 2-3 days later it becomes quite prominent and appears as chlorosis along the veins. Later green vein-banding occurs and is prominent on almost all the leaves.

Solanum nodiflorum. In about 15 days after inoculation leaves begin to show slight mottle along the veins. This later appears as distinct veinal mossic characteristic of this virus

mosaic characteristic of this virus.

Petunia hybrida. The first symptom of infection which appears about 16 days after inoculation is in the form of vein-clearing of youngest leaves. Darkening of the green colour along the veins is a subsequent development and is usually quite distinct. Leaves may at times show some distortion.

Datura stramonium. Efforts to transmit the disease to this host by mechanical inoculation were unsuccessful. It was made sure by back inocula-

tions to Nicotiana tabacum that the disease was not carried.

Potato variety President. Infection of this host takes place about 12 days after inoculation when small necrotic spots appear on or near the veins. These later develop into streaks along the veins on the under surface of the leaf followed by distinct veinal necroses. The necroses then progress towards the petiole and finally result in leaf-drop streak (Plate X, fig. 4).

## PROPERTIES OF THE VIRUS

Thermal inactivation. Exposure of the virus for 10 minutes in a water bath at various temperatures shows that the virus rapidly begins to lose activity after exposure to a temperature of 50°C. and at 54°C. the virus becomes innocuous.

Toleration to dilution. Inoculation of young tobacco plants with freshly extracted juice from diseased plant leaves as well as with juice diluted with sterilized distilled water showed that the infectivity begins to fall at a dilution of 1:100 and the virus is rendered innocuous at a dilution of 1:1000.

Longevity in vitro. Standard extract of the virus stored at laboratory temperature (29°-34·5°C.) was rendered innocuous after 24 hours whereas when the extract was stored at a lower temperature (8°-10°C.) it remained active for 32 hours and completely lost its infectivity after 36 hours.

The results of the experiments are summarized in Table I.

Table I
Properties of the virus

Thermal inac	ctivation		Toleration to	dilution		Longevity i	n vitro	
	No. of plants			No. of plants			No. of plants	
Exposure tempera- ture °C.	Inocu- lated	Infect- ed	Dilution	Inocu- lated	Infect- ed	Storage period	Inocu- lated	In <b>f</b> ect
Unheated control . 40	3 3 4 4 4 4 4 4 4	3 3 3 2 2 2 2 1 0 0	Nil (Control) 1:10 1:50 1:10 1:50 1:100 1:200 1:500 1:1000 1:1000	3 3 3 3 3 3 3 3	3 3 3 2 2 2 1 0	Fresh standard extract	4 4 4 4 4 4 4 4 4	4 4 2 3 0 0 0 0 0 0

Filterability. Standard extract prepared from the young infected tobacco leaves was filtered through Chamberland filters of different grades  $(L_1-L_5)$  and it was found that the extract after passage through the candles had been rendered inactive. The

filteration was carried out under reduced pressure of 1/5 atmosphere.

The reactions on differential hosts and the properties of the virus show that it is identical with Solanum virus 2 (Potato virus Y). The occurrence of this

virus in India was once reported by Pal [1943] as a result of the examination of diseased Phulwa plant tubers by Salaman in England. However, the occurrence of veinal necroses on Phulwa plants, an important diagnostic character of this virus on certain potato varieties, was not observed.

#### SUMMARY

The properties and reactions on differential hosts of a virus isolated from potato plant of Phulwa variety are reported.

The virus rapidly begins to lose activity after exposure to 50°C, and at 54°C, the virus becomes

innocuous. The virus is also rendered innocuous at a dilution of 1:1000 and after storage at room temperature for 24 hours. It is held back during passage through Chamberland filters (L<sub>1</sub>-L<sub>5</sub>).

The virus is identical with Solanum virus 2.

## ACKNOWLEDGEMENT

Thanks are due to Dr G. Watts Padwick, Imperial Mycologist, for his keen interest in the investigation.

#### REFERENCE

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## THE CYTOLOGY OF CARICA PAPAYA LINN.\*

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(Received for publication on 4 September 1944)

(With Plate XI and 53 text-figures)

A THREE years' scheme financed by the Imperial Council of Agricultural Research for a preliminary investigation on the cytology of papaya, with a view to a subsequent study of sex inheritance, was started at the College of Agriculture, Poona, in July 1940. The main object of this scheme was to study the cytology of the different sex-types in papaya with a view to find out whether there are any visible chromosomal differences in morphology or behaviour during cell division, which could be correlated with sex expression. The present paper describes the findings of the above investigation.

## THE GENUS CARICA

The botanical relationship of this genus has been the subject of much discussion and it has previously been classified under the families Passifloraceae, Cucurbitaceae, Bixaceae and Papayaceae, though it is now placed under Caricaceae along with the genus Jacaratia. The genus Carica comprises about 40 species all of which are indigenous to the American tropics, and are described as dioecious, except the cultivated species C. papaya, which is characterzied by a variety of sex types possessing various flower types. Judging from the chromosome number of the seven species hitherto investigated, C. chrysopetala, C. pentagona and C. candamarcensis [Heilborn, 1921], C. quercifolia [Storey, 1941], C. pubescens [Kumar and Abraham, 1942], C. dodecaphylla [Kumar and Srinivasan, 1944], and C. papaya (reported in this paper and in previous studies), it

appears that the genus Carica is characterized by a uniform chromosome number of 2n=18.

## PREVIOUS WORK ON CARICA PAPAYA

The earliest contributions to our knowledge of the histology of Carica papaya were made by Usteri [1907] and Kratzer [1918] both of whom have studied the development of the embryo-sac. Usteri [1907] found that the micropylar cell of the tetrad gave rise to the embryo-sac, while Kratzer [1918] observed that any cell of the tetrad might function to give rise to the embryo-sac. In 1921, Heilborn made one of the first important contributions to the cytology and embryology of a few species of Carica. He examined cytologically four species of Carica, namely, C. chrysopetala, C. pentagona, C. candamarcensis, and C. papaya, in all of which he found the diploid number to be eighteen. Heilborn [1921] describes the development of the embryo-sac in C. papaya which is rather out of the normal; the megaspore mother cell divides, but no cell wall is formed and consequently no tetrad formation takes place. Both nuclei divide again to give four nuclei and of these four, one divides again. Thus an embryo-sac is produced with five nuclei, three of which form the egg apparatus, while the remaining two nuclei may be considered as polar nuclei, though no fusion of these two nuclei was observed. After fertilization, a non-cellular endosperm is developed and later the egg-cell divides and gives rise to the embryo. In C. chrysopetala, a parthenocarpic species lacking male flowers, Heilborn [1921] observed a bi-nucleate embryo-sac, each nucleus having nine chromosomes. He has also observed quite

<sup>\*</sup> Findings of the investigation into the cytology of Carica papaya Linn., a scheme financed by the Imperial Council of Agricultural Research

large ovules with no archesporial cells, ovules with degenerating embryo-sacs and many ovules with well-developed embryo-sacs. In C. pentagona, another parthenocarpic species lacking male flowers, normal reduction divisions were observed by Heilborn [1921] in some ovules. Reduction division in C. candamarcensis and C. papaya was found to be quite normal and in a single embryo-sac mother cell of C. papaya he had seen quite normal diakinesis. Meurman [1925] in a study of the chromosome behaviour of some dioecious plants with reference to the sex chromosomes has recorded the presence of nine gemini in the pollen mother cells of Carica papaya and the absence of any sex chromosome pair in them. Sugiura [1927] in a study of the meiosis of pollen mother cell of *C. papaya* confirms Meurman's [1925] observations besides reporting the formation of a multipolar spindle. Various investigations carried out by Sakarai [1929] to correlate sex with external characters of seedlings gave negative results. In 1930 Lindsay investigated C. papaya and came to the conclusion that no evidence could be found for an unequal pair of chromosomes in the division of the pollen mother cells. Asana and Sutaria [1929] in a study of the development of pollen in C. papaya found the meiotic chromosome number to be nine and also reported the occurrence of multipolar and tripolar spindles. Agharkar and Banerji [1930] have traced the development of embryo-sac in C. papaya. They found that the chalazal cell of a linear tetrad of megaspores gives rise to the mature 8-nucleate embryo-sac. Genetical studies in C. papaya of great importance were carried on independently by Hofmeyr [1938] at Nelspruit, S. Africa, and by Storey [1941] at Hawaii, on the inheritance of sex-forms and they came to identical conclusions. These studies have shown that (1) sex-determination in papaya could be explained in terms of simple Mendelian factors, (2) the female represents a homozygous recessive genotype, while the male and the hermaphrodites are enforced heterozygotes, (3) all combinations of homozygous dominant geno-types are lethal to the zygotes, (4) sex determination may be said to be of the XY type (i.e. male and bisexual XY; female XX), and (5) fertilization is entirely at random and there is neither differential selectivity nor differential viability among gametes. Linkage studies have shown that the sex factors for the different types occupy the same locus on homologous chromosomes along parts of which crossing over occurs freely. Their [Hofmeyr, 1938 and Storey, 1941] cytological observations led them to the conclusion that no heteromorphic pair of sex chromosomes is to be found in papaya. Storey [1941] in a bulletin gives a summary of all the work done up to 1941 on papaya in Hawaii, where investigations have been actively pursued for the past 30 years, and also his findings on the cytology and genetics of C. papaya. The different sex types and flower types observed in C. papaya have been fully described in a recent publication [Kumar and Abraham, 1942].

## MATERIAL AND METHOD

Papaya plants grown in India are usually from seeds of non-descript varieties, sometimes named after the locality of its origin, but in most cases of a mixed and unreliable type. On this account, as well as to make the study as comprehensive as possible it was felt necessary to obtain seeds from reliable sources in various countries and grow them in Poona for collecting material for cytological studies. Requests for seeds were accordingly sent to Agricultural Stations of most of the tropical and sub-tropical countries, where papaya is cultivated. The response to this was very good and more than 100 samples of seeds of different varieties were obtained. Fifty of these varieties selecting at least one sample from each country were raised in Poona. Some of these produced interesting sex forms and all material for this work was obtained from these plants. The object in growing such a large number of varieties was to find whether geographic isolation and effect of various climatic conditions have affected the chromosome make-up of the species, the original home of which is known to be South America, from where it has spread to the various tropical and sub-tropical countries in the world during the last three centuries.

Considerable difficulty was experienced in the beginning in obtaining good cytological preparations by using the common fixatives and staining methods. This was found to be mainly due to the presence of a large quantity of easily coagulable latex in the cells of even the floral parts. Attempts at dissolving the latex before or during fixation interfered with proper fixation. After trials with several chemicals, it was found that a modification of Bouin's fluid at a temperature of 35°C., applied after pre-treating the material for 2-3 minutes in Winge's Picro-carnoy, also at the same temperature, gave quite satisfactory results. Staining was mostly done by the iodine-gentian-violet method. But as this stain though unsurpassed for clarity and transparency of chromosome figures tends to fade rapidly after about six months, several new modifications of staining have been tried. One new method which gave good preparations is described below.

The slides are brought to water and then mordanted for about an hour in a mixture of equal parts of five per cent ferric ammonium sulphate and five per cent ferrous ammonium sulphate; then washed in running water for 10 minutes and stained in haematoxylin made according to Earle's [1939] formula, for about five minutes; then destained

in the first mordant diluted with equal part of water till the cytoplasm is clear and the chromosomes are stained only a light bluish black; next, the slides are washed in running water for about 15 minutes. After completing this abbreviated schedule of haematoxylin staining, the slides are stained with iodinegentian-violet in the usual manner. This superimposing of gentian-violet stain on a light haematoxylin background gives a much better preparation than either of the two stains applied alone, particularly for prophase stages which are difficult to stain in plants in which chromosomes are very small. It has been noted that some preparations stained early in 1941 by this schedule still retain clearly the stain after three years.

For the detailed study of the morphology of somatic chromosomes, Lewitsky and others found that a fixative without acetic acid followed by the Feulgen staining was the best. This was particularly so in plants with small chromosomes. As in papaya the chromosomes are very small, it was thought that application of this method would reveal the details of morphology of the chromosomes more clearly than other methods. attempts at trying Lewitsky's methods failed to give satisfactory preparations and so the common fixatives containing acetic acid in various propor-tions had to be used. While this followed by gentian violet staining gave good results for study of number and gross morphology of chromosomes, the finer details were not always clear. Another method which gave good preparations may be mentioned here. Root-tips were fixed in Lewitsky's chrom-formalin mixture. The slides were taken down to 70 per cent alcohol and left overnight in a jar of 70 per cent alcohol to which a little lithium carbonate had been added. They were then brought down to water and stained with crystal violet for about two hours and then destained in the usual way.

Anthers of correct stages were smeared and fixed in Belling's Navashin for two hours, after which they were washed, taken up to 70 per cent alcohol and left overnight in 70 per cent alcohol with a little lithium carbonate. They were then stained in crystal violet as described above. This method gave particularly good preparations in which large number of p.m.c.s with Metaphase I plates were obtained. Both side views and polar views of first metaphase were obtained. In the side view of Metaphase I plates, the chromosome complement of a number of pollen mother cells could be clearly analysed and the polar view of M-I plates exhibited very clearly various types of secondary association. These methods were supplemented with acetocarmine smears, which facilitated examination of a large number of plants.

For the study of megasporogenesis, slices of ovaries of different stages of development were fixed either in hot corrosive sublimate, formalin-acetic-alcohol or in Zenker's fluid after a minute's prefixation in Winge's picro-carnoy. They were sectioned at thicknesses varying from 8  $\mu$  to 14  $\mu$  and stained in Haidenhain's iron-alum haematoxylin.

In Table I is given a list of the different varieties of papaya that have been examined cytologically and their source, and also the chromosome numbers of two other species of *Carica* namely *pubescens* and *dodecaphylla*.

#### OBSERVATIONS

The meiosis in the pollen mother cells of twentyone varieties of papaya have been examined by permanent preparations. Meiosis of three of these (variety Nos. 15, 46 and 99) have been studied in the hermaphrodite also; while in one-variety No. 46-meiosis has been studied in detail in all the three sex types, viz. male, female and hermaphrodite. In addition to these, a few more varieties have been examined both in the male and the bisexual through temporary aceto-carmine smears. Analysis of the chromosomes of these sex-types has shown that the haploid number is n=9 for all of them. Slight morphological differences among the bivalents have been observed. The varieties also showed slight variations in the size of the chromosomes. Apart from these minor variations, meiosis in both male and hermaphrodite was found to be normal. Comparison of the chromosomes of the male and the hermaphrodite showed no differences which could be correlated with the differences in sex-expression. Somatic chromosomes of twelve varieties have been examined and the number found to be 2n=18. Here also distinct morphological differences in the chromosomes have not been observed. All the chromosomes have median or sub-median constrictions. The length of somatic chromosomes at metaphase varies from 2 to 3  $\mu$ , while the length of meiotic chromosomes at metaphase in the pollen mother cells (as determined from enlarged photomicrographs) is about one-tenth of the length at somatic metaphase.

One variety of Carica papaya (variety No. 98), seeds of which were got from Chengannur in Travancore State, is the most distinct in respect of its vegetative characters. It is characterised by the presence of yellow colour in all parts of the plant. Fruits are bright yellow from the early stages. Only the leaf blades have a greenish colour mixed with yellow. There are no male plants in this variety. Examination of the pollen mother cell in the hermaphrodite showed that the chromosomes number in this variety also is n=9. Reference to literature (which is by no means comprehensive) shows that this is an undescribed variety.

Mitosis. It has been observed that when seeds from fruits of female plants growing in close proxi-

Table I

A list of different varieties of papaya examined

No.	Varietal name	Locality	Source	Chrom N	
				n	2n
$\frac{1}{2}$	Madhubindu Alipore	Kathiawar Alipore	Ramji Hansraj & Sons, Kathiawar Secretary, Royal Agri-Horticultural Society, Calcutta	9 9	18 18
3 4 5 6 8 9	Honeydew	Baroda Coimbatore . Kodur . Kodur Kodur . Kodur . Malaya	Horticulturist to Government, Baroda State Superintendent, Central Farm, Coimbatore . Sri K. C. Naik, Kodur Farm	9 9 9 9 9	18 18 18 18 18 18
15 29 34 40 44 46	Washington . Solo	Poona Netherlands S. America S. Africa Saravak Formosa	J. D. J. Hofmeyr, Nelspruit, S. Africa.  Dr Matsuara, Hokkoide, Imperial University, Sapporo, Japan	9 9 9 9	18 18 18
47 54 55 56 98	Heito-Delicious Kuching Kuching Kuching Marumi ogata Yellow papaya	Japan	Ditto Dr W. B. Storey, Hawaii Ditto Dr H. Matsuura, Japan Mr A. T. Abraham, Chengannur, Travancore State	9 9 9 9	
99 105	Solo papaya Florida green Carica pubescens Carica dodecaphylla	Australia	Dept. of Agriculture, Queensland Director of Agriculture, Guiana	9	18 18

mity to male plants are sown, the resulting progeny shows an approximately equal proportion of female and male plants with a few hermaphrodites. So a study of the somatic chromosomes in the roottips of a number of young seedlings should show whether there is any difference between the chromosome make-up of the male and the female. With this object in view, seeds from a single fruit (of variety No. 34) obtained by open pollination were sown and the tips of the main root in about 100 seedlings were fixed. Examination of the metaphase chromosomes from a number of sections showed that the differences from root-tip to root-tip were not greater than those observed from cell to cell in the same section. Figs. 16 to 18 show somatic metaphases from different root-tips. Unless there is a marked difference between chromosomes of one sex and another, a study of this type may not be expected to reveal any differences that may exist. This is particularly so in a case like papaya where the chromosomes are extremely small in size. However, this study shows that no marked diffrences exist between the chromosomes of the male and the female in the same variety. Further, from independent genetical studies Hofmeyr [1938] and Storey [1941] came to the identical conclusion that in papaya male and hermaphrodite are heterogamous for sex, while the female is homogamous. So, if at all a heteromorphic pair of chromosomes carrying the genetic factors for sex is present, it should be expected in the male or hermaphrodite and not in the female.

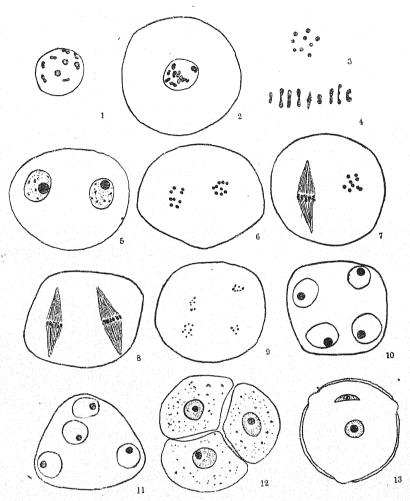
As previously stated, variety No. 46 was selected for a critical and comparative study of meiosis in male, female and hermaphrodite sex-types, with a view to settle once for all the question whether there are any visible chromosomal differences in morphology or behaviour during cell division which could be correlated with sex expression.

Meiosis in hermaphrodite of variety 46. In the hermaphrodite sex-type there are only five stamens, while in the male, there are ten stamens.

In very young anthers, the pollen mother cells remain packed together closely and are in close contact with the tapetal cells which form a ring around them. They begin to round off at about the stage of diakinesis. In aceto-carmine smears

also, this is the earliest stage at which clear visibility of chromosomes is possible. The diakinetic stage is preceded by a 'diffuse stage' when the chromosomes are not stained properly. This observation was made from a large number of permanent preparations, in which the prophase and stages after diakinesis are well-stained, whereas the stage immediately preceding diakinesis is not stained. Such a feature has been noted in other plants also.

At late-diakinesis, the eighteen chromosomes which form nine pairs are distributed along the periphery of the nucleus (Fig. 1). The nucleolus has disappeared at this stage. The bivalents themselves may be either of the rod-type or of the ring-type. After examination of a large number of diakinetic stages, it was found that of the nine bivalents, one to three are of the ring-type, while the rest are all rod-shaped.



Figs. 1-13—Carica papaya—Hermaphrodite plant of variety No. 46

- Diakinesis in pollen mother cell. 3 bivalents are ring-shaped while the remaining 6 are of the rod-type. ×1667. Fig.
- Prometaphase of meiosis. Fig. 2.
- Polar view of metaphase I. ×1667. Fig. 3.
- Lateral view of metaphase I. ×1667.
- Fig. 5. Interkinesis nuclei. ×1167.
- Metaphase II. Both the metaphase plates in Fig. polar view. ×1167.

  Fig. 7. Metaphase II. One of the metaphase plates in

- polar view while the other shows the side view.
- ×1167. Metaphase II. Both the metaphase plates in Fig. 8.
- side view.  $\times 1167$ . Telephase of second division.  $\times 1167$ . Fig. 9.
- Iso-bilateral type of pollen tetrad formation. Fig. 10.  $\times 1167.$
- Tetrahedral type of pollen tetrads.  $\times 1167$ . Fig. 11.
- Frg. 12.
- The young microspores. ×1167.
  Two-celled microspore at the time of shedding. Fig. 13.  $\times$ 800.



During the next stage of prometaphase (Fig. 2) the bivalents undergo a converging movement, until they are in close assemblage in the centre of the nucleus. Metaphase follows prometaphase. The nine bivalents are arranged on the equatorial plate (Fig. 3). A characteristic feature of metaphase is the association or approximation of the different bivalents. This secondary association which is the pairing of ancestrally related bivalents has been studied in detail in the male of this variety. Some evidence is available to support the findings of earlier investigators that metaphase is a very transient stage, as numerous cells with the bivalents all arranged in one plane are not met with. But in some cases such arrangement in one plane has been met with and in these secondary association is unmistakably evident. The size of the bivalents varies from 0.2 to 0.3  $\mu$ . Fig. 4 shows the side view of the nine bivalents at metaphase I. The separation of the bivalents during anaphase is uniform and regular, though very occasional lagging on spindle has been observed. At the end of the first anaphase, two interkinesis nuclei are formed (Fig. 5).

Sugiura [1927], Lindsay [1930] and Asana and Sutaria [1930] have reported the presence of multipolar spindles. Occasionally it has been met with in the present study also.

The second division is quite normal and nine chromosomes have been counted in both metaphase plates in the same pollen mother cell (Fig. 6). The chromosomes are smaller than in the first division and any difference in size apparent in the first division is not seen at the second. Fig. 7 shows a second metaphase plate, where one of the groups is in the polar view while in the other the side view is seen. Secondary association persists in second metaphase also. The further meiotic process (Figs.

8 and 9) is regular resulting in tetrads, which may be either tetrahedral (Fig. 11) or isobilateral (Fig. 10). Wall formation is by furrowing (Fig. 12) as already reported by Asana and Sutaria [1928]. The mature pollen grain at the time of shedding is bi-nucleate (Fig. 13).

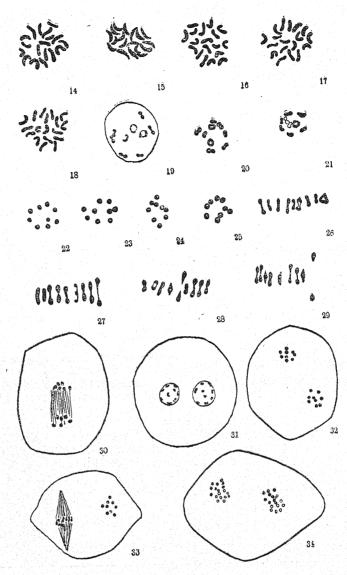
Meiosis in male of variety 46. At early diakinesis nine bivalents are observed distributed peripherally along the nucleus (Fig. 19). The nucleolus is seen very faintly stained. Fig. 20 shows late diakinesis. Two of the bivalents are of the ring-type, while the remaining seven are rod-shaped. At diakinesis, the two chromosomes of a bivalent could be clearly seen and careful comparison of the size and shape of the two members of each pair revealed no inequality between the chromosomes of any of the pairs. During pro-metaphase (Fig. 21) bivalents are in close assemblage in the centre of the nucleus. At metaphase the bivalents again are separated and arrange themselves on the equatorial plate (Fig. 22). The characteristic feature of metaphase is the association or approximation of the different bivalents. This secondary association is prevalent in the majority of pollen mother cells. Out of 62 first metaphase plates examined, 44 exhibit secondary association, while the rest do not. The paired The paired bivalents are similar in size and configuration. A variable number of bivalents are seen to be secondarily associated. Fig. 22 shows the stable configuration, which consists of one bivalent in the centre with the remaining eight arranged in a ring around it. Figs. 23 to 25 and photomicrographs (Plate XI, figs. 1 and 2) show various degrees of secondary association. This pairing is exhibited very clearly at the first and second metaphase.

Table II gives a summary of the various types of secondary association.

TABLE II

A summary of various types of secondary association

No. of		No. of bivalents	No. of cases	Total		
associations	1	2	3	4		
1 2	7 5 5 6 6	1 2	i.		24 11 3	24 · 14
3	$\begin{cases} 3\\4\\5 \end{cases}$	3		i	$\begin{bmatrix} 3\\1\\1\\1\end{bmatrix}$	5
4	1	<b>4</b> 33 5 6			1	1 Total 44



Figs. 14-34

- Fig. 14. Metaphase plate from root-tip of C. dodecaphylla.  $2n=18. \times 2167.$
- Fig. 15. Metaphase plate from root-tip of C. pubescens. 2n=18. ×2167.
- Figs. 16-22.—Carica papaya (male plant)
  Figs. 16-18. Metaphase plates from root-tips of different seedlings of C. papaya (Variety No. 34). 2n=18.  $\times 2167$ .
- Two of the bivalents are ring-Fig. 19. Early diakinesis. shaped.  $\times 1667$ .

- Fig. 20. Late diakinesis. ×1667. Fig. 21. Prometaphase. ×1667. Fig. 22. Metaphase I. One bivalent is in the centre with
- eight others arranged around it. ×1667.

  Figs. 23-25. M-I plates showing different degrees of secondary associations. Fig. 23: 1(2)-7(1); Fig. 24:
- 2(2)-5(1); Fig. 25: 4(2)-I(1); (maximum association). All. ×1667.

  FIG. 26. Side view of M-I. ×1667.

  FIG. 27. Same as above. Note the bivalent on the extreme right about to segregate. ×1667.

  FIG. 28. One bivalent separating precociously. ×1667.

  FIG. 29. Same as above showing a later stage. The segregating chromosomes are similar. ×1667.

  FIG. 30. Anaphase I. ×1167.

- Interkinesis nuclei.  $\times 1167$ . Fig. 31.
- Metaphase II. Polar view. Two groups of nine chromosomes each are seen. ×1167. Fig. 32.
- Same as above. One of the plates in side view. Fig. 33. ×1167.
- Fig. 34. Anaphase II. Four groups of nine chromosomes each are seen. '

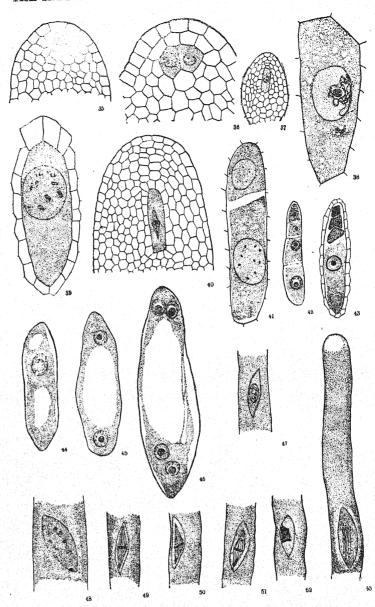
In computing the number of secondary associations in each case, an association of four bivalents into one group is counted as three, that of three bivalents as two, and that of two bivalents as one. The number of secondary association per plate ranges from one to four, the mode being one which occurs in 24 out of 44 cases. The maximum of four associations was seen once in 44 plates examined. It shows four groups of two bivalents each and a single bivalent, on the whole making five separate associations (Fig. 25). Subsequently, another case of a maximum of four associations was met with, which showed one group of three bivalents, two groups of two bivalents each, and two unassociated bivalents (Plate X1, fig. 2).

A large number of metaphase plates in lateral view were examined. The outline of all the nine bivalents is clearly distinguishable in side view and Figs. 26 and 27 represent two such metaphase plates. The bivalents have been separated laterally while drawing. This fact namely that all the nine chromosome pairs are distinguishable on a number of spindles in lateral view makes possible the definite conclusion that in papaya there are no morphological differences recognizable by methods at present available between the two chromosomes of any pair in a pollen mother cell. In very early anaphase stages, the spindle fibres have already arranged themselves into a bipolar spindle, and the two members of each bivalent begin to be drawn apart. In such stages where the chromosomes of the different pairs are just beginning to separate, it was often found that in one particular bivalent, the paired chromosomes have already separated and are well on their way to the poles (Figs. 28 and 29). Plate XI, fig. 3 shows clearly one such plate, where, while the eight bivalents are still on the equatorial plate, one bivalent, however, has separated, the chromosomes of which are about half way through on their way to the poles. This interesting phenomenon was observed in about 10 cases out of about 15 of exactly the same stage examined. This precocious anaphasic separation of one of the bivalents is interesting, because, such a precocious separation during early anaphase is a characteristic feature of the sex-chromosome pair. This has been found by Sinoto [1928] to be a common feature of the sex chromosomes of the dioecious species in both dicotyledons and monocotyledons that he had studied. This phenomenon has again been recorded in Coccinia indica by Kumar and Deodikar [1940]. After a critical examination of the chromosomes that separate precociously in all the 10 cases reported, it can be said that there are no visible morphological differences between the chromosomes of the bivalent that exhibit precocious separation.

In second metaphase plates, two groups of nine univalents each could be clearly seen (Fig. 32).

Secondary association persists in anaphase I and metaphase II. The subsequent stages in meiosis leading up to the formation of tetrads are quite normal and similar to that described already in the case of the hermaphrodite plant.

Microgametogenesis. Pollen grains from the male plant of variety No. 46 were germinated on a agarsugar culture medium and the nuclear division of the generative cell in the pollen tube was studied. The method employed is the one described by Maheshwari and Wulff [1937]. The mature microspore at the time of shedding is spherical, two-celled and possesses three germpores (Fig. 13). After preliminary trials, it was found that germination of the pollen grains was best in a culture medium consisting of agar and sugar in the ratio of 1:10. For getting the requisite growth in length of the pollen tube, after which the generative cell begins to divide, it was found necessary to allow the microspores to germinate in the culture medium for about twenty hours. The slides were fixed in Formalin-Acetic-Alcohol for about three hours and stained in iron alum haematoxylin. The pollen tube begins to grow from one of the germ pores and as it goes on elongating, the crescent shaped generative cell moves down into the pollen tube and is about 6 µ distant from the free end of the pollen tube, when it begins to divide (Fig. 53). The pollen tubes showing division stages are about 40  $\mu$  in length. The tubes are comparatively broad, the breadth being about 1 \(\mu\). In Fig. 47 is represented a pollen tube with the nucleus of the generative cell in the prophase of division. Figs. 49 and 50 show lateral views of the metaphase stage. The generative cell usually lies in the middle of the pollen tube though occasionally it may be close to one wall (Fig. 50). Plate XI, fig. 4 is a photomicrograph of a similar stage. The cytoplasm of the generative cell is differentially stained from that of the tube and the outline of the generative cell stands out markedly from the cytoplasm of the tube. Fig. 48 shows a polar view of the metaphase. Nine chromosomes are seen. No differences are noticeable between the chromosomes. The frequent arrangement of chromosomes in a line, one below the other, is not met with in the present case, as apparently this type of spatial distribution of chromosomes is necessary only when the chromosomes are relatively large as in Lilium and other genera [O'Mara, 1933]. Since in the present case, the tube is broad and the chromosomes are rather small, there is no necessity for such a linear arrangement of the metaphsae chromosomes and so they arrange themselves in a plate in the normal way. In Fig. 51 is seen what appears to be an unseparated chromosome lying outside the plate. Anaphase is normal and is shown in Figs. 52 and 53. The later stage showing the organized male gametes was not observed.



Fras. 35-53

- Figs. 35 to 46 of *C. papaya* (female plant) Single hypodermal archesporial cell. ×380.
- Frg. 35.
- A plate of two archesporial cells with a layer of wall cells. ×600. Frg. 36.
- Megaspore mother cell with about five layers of Frg. 37. parietal tissue. ×250.
- M.M.C. with the nucleus in the prophase stage. Frg. 38.  $\times 1250.$
- M.M.C. with the nucleus in diakinesis of the hetero-Fig. 39. typic division; nine bivalents can be counted. ×1250.
- Fig. 40. M.M.C. deeply embedded in the nucellus with its nucleus in metaphase (side view) of the hetero-
- typic division. ×550.

  Dyad with the two nuclei about to divide again. Frg. 41.
- ×1250.
  A linear tetrad of megaspores. ×600.
  Same as above, showing the functional chalazal megaspore and the three degenerating megaspores. ×600. Fig. 42. Fig. 43.

- Figs. 44-46. One, two and four nucleate embryo-sacs respectively.  $\times 1250$ .
- Figs. 47 to 53 of *C. papaya* (male plant)

  Fig. 47. Spindle-shaped generative cell in pollen tube, with its nucleus in the prophase stage. ×875.

  Fig. 48. Metaphase stage in the division of the generative nucleus. Nine chromosomes are seen. ×1250.
- (Figs. 49 to 53 magnified 875 times)

  Fig. 49. Lateral view of metaphase stage. The generative cell lies in the middle of the pollen tube
  Fig. 50. Same as above. The generative cell lies close to one wall of the pollen tube.
- Same stage as above. One of the chromosomes Fig. 51.
- lying outside the plate.

  Anaphase of the division of generative nucleus.

  Same as above showing the distance of the genera-Fig. 52.
- Fig. 53. tive cell from the tip of the pollen tube.

Meiosis in female of variety 46. In Carica papaya there are numerous anatropous ovules that arise not only from the placental region, but sometimes also from the entire inner wall of the ovary. All the ovules do not develop to maturity. A good number of the ovules degenerate in the early stages of their development. In very young ovules, a single primary archesporium is differentiated as a hypodermal cell, which is larger and takes a deeper stain than the other cells of the ovule (Fig. 35). In a solitary case, however, a plate of two archesporial cells with the primary wall cells already cut off was noticed (Fig. 36), though no case of two megaspore mother cells was observed in the later stages in the development of the archesporial cells into embryo-sacs. The primary archesporium cuts off a parietal or wall cell and functions directly as the megaspore cell. As the ovule develops, this wall cell by repeated periclinal divisions forms a parietal tissue of about 6 to 8 cells in thickness. As a result of the formation of this thick parietal tissue the megaspore mother cell is pushed deep into the nucellus. By this time, the megaspore mother cell has increased in size considerably. Just when the archesporial cell has cut off the parietal cell, the inner of the two integuments arises from the side of the ovule. The initiation of the outer integument follows in close sequence and it grows more rapidly than the inner integument which is completely enveloped by the outer integument when fully developed. The integuments are 3-4 cells thick. Both the integuments take part in the formation of the micropyle. Fig. 38 shows a fully enlarged megaspore mother cell with its nucleus in the prophase stage. In Fig. 39 is observed a megaspore mother cell nucleus in the diakinesis stage of the hetero-typic division. Nine bivalents are clearly observed at this stage. The bivalents are distributed peripherally in the nucleus and are therefore in different foci. About 10 such stages of the megaspore mother cell nucleus in diakinesis were observed. A careful examination of all of them showed no difference between the chromosomes of any of the pairs. Their behaviour during meiosis also seems to be quite normal. Preparations showing side views of the meiotic metaphase were available (Fig. 40). In such side views, all the nine bivalents could not be identified though the profile of about 5 or 6 of the bivalents could be made out clearly. Anaphasic separation is quite normal and the two daughter cells of the resulting dyad (Fig. 41) again divide to give rise to a linear tetrad of megaspores (Fig. 42). Invariably, in all cases, it was the chalazal megaspore that was functional while the rest degenerated (Fig. 43). The functioning megaspore which is full of cytoplasm enlarges in size considerably to form the uninucleate embryosac (Fig. 44). The single nucleus divides. The two

dauther nuclei move towards the poles of the embryosac and a central vacuole is formed (Fig. 45). Another division of these two nuclei gives rise to a four-nucleate embryo-sac (Fig. 46). Further stages in the development of the mature embryo-sac were not studied. The development of the embryo-sac so far described is in agreement with that already found by Agharkar and Banerji [1930].

## Discussion

Secondary association. The occurrence of marked secondary association of the bivalents in papaya has been recorded for the first time in the present investigation. About 70 per cent of metaphase I plates exhibit secondary association. From Table II, it is evident that the most frequent association is into eight groups (one group of two bivalents and seven separate bivalents), while the maximum association observed in a solitary case is into five groups (four groups of two bivalents each and one single bivalent). The criterion for deducing the basic chromosome number of a genus from secondary association, is the maximum association observed. In the present case, the maximum association is into five groups, and as such the basic chromosome number would be five for the genus Carica. In this connection, a detailed study of the other species of Carica may throw more light on the question of the basic chromosome number of this genus.

The cytological basis of sex expression. The discovery of a morphologically distinguishable difference between the chromosome complements of the male and female in animals, where generally the two sexes are found on separate individuals, was followed by the finding that sex-linked genes are located on these extra or accessory chromosomes, which came to be known as sex-chromosomes as distinguished from the autosomes, which are identical in both sexes. A similar cytological situation was found in a number of dioecious flowering plants also. In a large number of investigated cases including many species of insects, most of the mammals, and in several dioecious plants, the females have two X chromosomes, the male but one. In most of these, the males have another chromosome, the Y chromosome, but in some instances, this is absent. Thus the female is XX or homogametic, while the male is XO or XY and heterogametic. While this is the common condition, variations from this are met with in a number of cases. The occurrence of 'intersexes', in the moth Lymantria dispar, led Goldschmidt [1934] to propose a modification of the simple explanation of sex determination described above. According to his 'balance theory of sex', sex development (male or female) is the result of a competition between opposed tendencies in which the final expression is determined by the relative strengths or balance existing between the factor for maleness and the factor for femaleness. A similar condition has been noted by Bridges [1925] in *Drosophila*.

The appearance in most species of animals of two sharply defined sexes means that there are probably two relatively stable points of equilibrium, one centering around maleness, the other around femaleness, and that in such cases the excess of one type of gene over the other is considerable and decisive in the earliest stages of development. The decision is usually given by the presence of two sex chromosomes in one set of individuals and by but one in the other. The role of a sex chromosome in determining sex is therefore like that of a weight added to either one side or the other of a scale; it tips the balance and only thus decides the outcome, and it probably does this by virtue of the specific genes which it carries [Sinnott and Dunn, 1939].

We shall now consider briefly the position regarding plants which have not been so adequately analysed. In most of the flowering plants, the same individual and generally the same flower bears both types of gametophytes, so that the sporophyte is hermaphroditic. In some species male and female flowers are borne on separate individuals and the question of sex determination arises only in these dioecious plants. But it must be noted here that even in strictly dioecious plants, unlike the case of animals, the differences between male and female individuals extend only to the floral parts. The chromosome mechanism of primary sex determination has been studied in a number of dioecious plants and found to be similar to that in animals. In most of these the female is homogametic while the male is heterogametic. Fragaria elatior [Lilienfeld, 1936] and Coccinia indica [Kumar and Deodikar, 1940] are the only two recorded cases of flowering plants in which the female is heterogametic. In many dioecious plants, however, cytological examination has failed to reveal any visible chromosome differences between the sexes.

In the genus Carica most of the forty species are dioecious, whereas the cultivated Carica papaya possesses in addition to the strictly male and female plants a number of 'intersexes'. The main object of the present investigation has been to study the cytology of the different sex-types in this plant with a view to finding whether there are any visible differences either in morphology or behaviour during cell division in the chromosome complements of these types which could be correlated with sexexpression. The negative findings of a few earlier investigators already mentioned were not of importance for this study as none of them had made a critical examination of the chromosomes of the three main sex types in the same variety. In the present investigation meiosis in the hermaphrodite and male of one variety has been described in detail.

Meiosis in the female of the same variety has also been studied in detail for the first time. The course of meiosis in the above three sex-types is normal. No visible differences in the chromosome morphology of the different sex types are evident, nor is there any difference in the behaviour of the bivalents in the hermaphrodite and female. In the male, however, an interesting behaviour of one particular bivalent during early anaphase has been observed. During early anaphase, one particular bivalent separates precociously, while the remaining eight are still on the equatorial plate. This particular bivalent has a single terminal chiasma. phenomenon has been observed in about 10 cases out of about 15 early anaphase stages examined. This interesting behaviour of a single chromosome pair during early anaphase has not so far been reported by any of the earlier workers on C. papaya. It may be that this bivalent which separates precociously during early anaphase may be the sex chromosome pair, carrying the genetic factors for sex. It has already been stated that precocious separation appears to be a common feature of the sex chromosomes. This has been reported in Coccinia indica by Kumar and Deodikar [1940]. Sinoto [1928] found this phenomenon to be a common feature of the sex-chromosomes of the dioecious species in both dicotyledons and monocotyledons that he had studied. But then in the present case the separating chromosomes are not a heteromorphic pair. They are quite alike one another. All that can be said then is that out of the nine bivalents, one behaves like a sex-chromosome in so far as precocious anaphasic separation is characteristic of the sex-chromosomes, there being no visible morphological differences between them.

Fortunately there is already data on the genetic basis of sex expression in Carica papaya. Hofmeyr [1938] in South Africa, and Storey [1941] in Hawaii, as a result of independent investigations on the sex inheritance of papaya, arrived at identical conclusions. According to these authors the male and hermaphrodite are heterogametic while the female is homogametic. So if an unequal pair of sex chromosomes is to be found it should occur in the male and hermaphrodite and not in the female. Due to the ease with which meiosis could be studied in pollen mother cells, the male and hermaphrodite have been more fully examined than the female. In neither of these was any difference between bivalents or between the two components of a single bivalent noted. Sufficient number of cases of diakinetic stage in meiosis in megaspore mother cell have been examined to know that there also no differences between the chromosomes of any of the pairs exist.

It may be mentioned in passing that due to variation in climatic factors, weakening of the plant due to manurial deficiency and other undetermined causes, some papaya plants which at the commencement of flowering produce female or bi-sexual flowers, later produce only male flowers. Such variation in sex in the same plant shows that the balance mechanism of sex in this plant is in a delicate equilibrium.

## SUMMARY

A comparative cytological study of three species of *Carica* and twenty-one regional types of *C. papaya* collected from different parts of the tropics was undertaken.

The chromosome numbers in all the cases studied is n=9, 2n=18.

A detailed and critical examination of the three main sex forms of a selected type shows that there are no differences in the morphology of the chromosomes of the hermaphrodite, the male and the female sex-types of the same variety. In the male papaya, however, the phenomenon of precocious anaphasic separation of one particular bivalent has been observed and it is suggested that this interesting feature is in keeping with the behaviour of a heteromorphic pair.

Study of the somatic chromosomes shows that there are no detectable differences between the chromosome complement of one sex type and another.

Examination of the chromosomes of the strictly dioecious species, *C. pubescens*, also shows that what might be called 'sex-chromosomes' are not present in this species also.

From the findings of the present cytological investigations, which is the first to include all the three sex types of a single variety, it appears that variation in sex expression in *C. papaya* is not associated with any visible morphological difference between the 'autosomes' and the 'sex-chromosomes'.

## ACKNOWLEDGEMENTS

In conclusion, the authors wish to express their sincere thanks to the Imperial Council of Agricultural Research for the financial aid given, which made this work possible, and to the various persons who kindly obliged them with seeds of the different species and varieties of papaya.

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# CRYPTOSTEGIA GRANDIFLORA R.Br., A WAR TIME SOURCE OF VEGETABLE RUBBER

## V. METABOLIC STUDIES\*

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(With Plate XII and one text-figure)

The object of the investigation was to study metabolic changes of the seedlings of Cryptostegia grandiflora with relation to their growth. The seeds after soaking in water were sown in the nursery and daily observations were made on 10 seedlings. The percentages of nitrogen, inorganic constituents and resins have been given but the estimation of rubber could not be carried out for reasons given in the experimental section and would be taken up again.

## EXPERIMENTAL

The seeds (1 oz. comprising about 3,000) of Cryptostegia grandiflora were soaked in water for 24 hours for presoaked seeds give on sowing a quicker and more uniform germination than dry seeds.

The soil of the nursery after manuring it with farmyard manure (eight cart loads per acre) was worked to a fine tilth and levelled by means of a levelling board. On 23 September 1943, the presoaked seeds were evenly spread over a flat bed (21 sq. ft.) with the hand. They were then lightly covered with soil by passing a hand rake over the bed, and were about  $\frac{1}{4}$ - $\frac{1}{2}$  in. deep in it. The surface of the bed was then gently evened out and the soil on the top was compacted by pressing by a light wooden roller. The nursery was then hand-watered and covered with gunny sheets (a mulch of bhusa about 1 in. thick would also serve the purpose). Thereafter it was hand-watered twice daily, morning and evening. The gunny mulch helped to conserve the moisture in the soil and produced a humid atmosphere necessary for the proper germination of When germination started on 27 the seeds. September 1943, gunny sheets were removed. In about a couple of days over 90 per cent of the seeds had germinated, the stand of the seedlings being quite uniform.

On the fourth day of sowing the seedlings showed differentiation into yellow cotyledons, white plemule

and roots. After one week the seedlings showed pink stems and green leaves. In the first fortnight there was only one pair of leaves, in the third week there were two and three pairs of leaves and two months old seedlings had five and six pairs of green leaves (Plate XII, fig. 1). The stems remained pink. For the analysis 10 seedlings were carefully dug out intact, washed with water from the adherent soil and the fresh weight was taken after drying them between folds of filter paper. The seedlings were kept over sulphuric acid for about two months when they were dried up completely and turned brittle. The dry seedlings were crushed and kept in a packet of filter paper and several of these packets were extracted at a time with alcohol to find out the amount of solubles or resins. The material of every packet was then dried at 90°C. in an air oven and from the loss it suffered during extraction the percentage of resins was calculated. The resin-free material was re-soxhleted with benzene for estimating its rubber content, but it showed no loss in weight and was further extracted with chloroform in an apparatus designed by the authors. The apparatus consisted of a cone made of wire gauze and was placed in the distillation apparatus on a triangular stand made of wire. This cone was fitted with a cone of filter paper in which several of the packets containing the material were kept for extraction (Plate XII, fig. 2).

After extraction for 36 hours the material of every packet was dried at 90°C. It did not suffer any loss in weight but, on the contrary, showed a slight increase. The rubber could not thus be estimated in the seedlings. The very fact that benzene and chloroform failed to extract rubber from the seedlings dried over sulphuric acid for two months was indicative of a change that rubber underwent; the latter became converted into such substances which are insoluble in rubber solvents. Inorganic constituents (ash) and total nitrogen of the seedlings were also determined but the quantity of the first 15 samples was not enough for estimating both and so only their nitrogen (by Kjeldahl method) was determined. The growth observations as well

<sup>\*</sup> Parts I and II of this series were published in the J. Indian Chem. Soc. (1944) and parts III and IV in the J. Indian Chem. Soc. Ind. and News Ed. (1945).

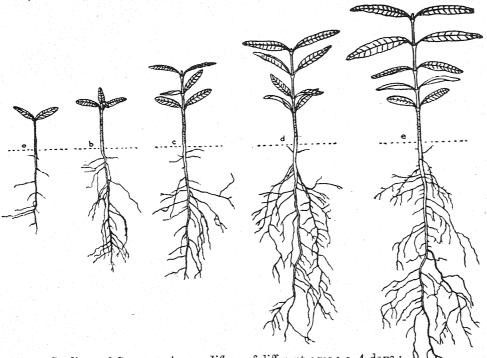


Fig. 1. Seedings of Cryptostegia grandiflora of different ages: a, 4 days; b, 11 days; c, 22 days; d, 36 days; e, 43 days

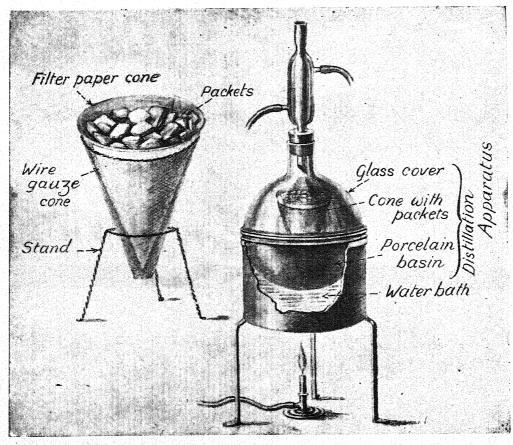
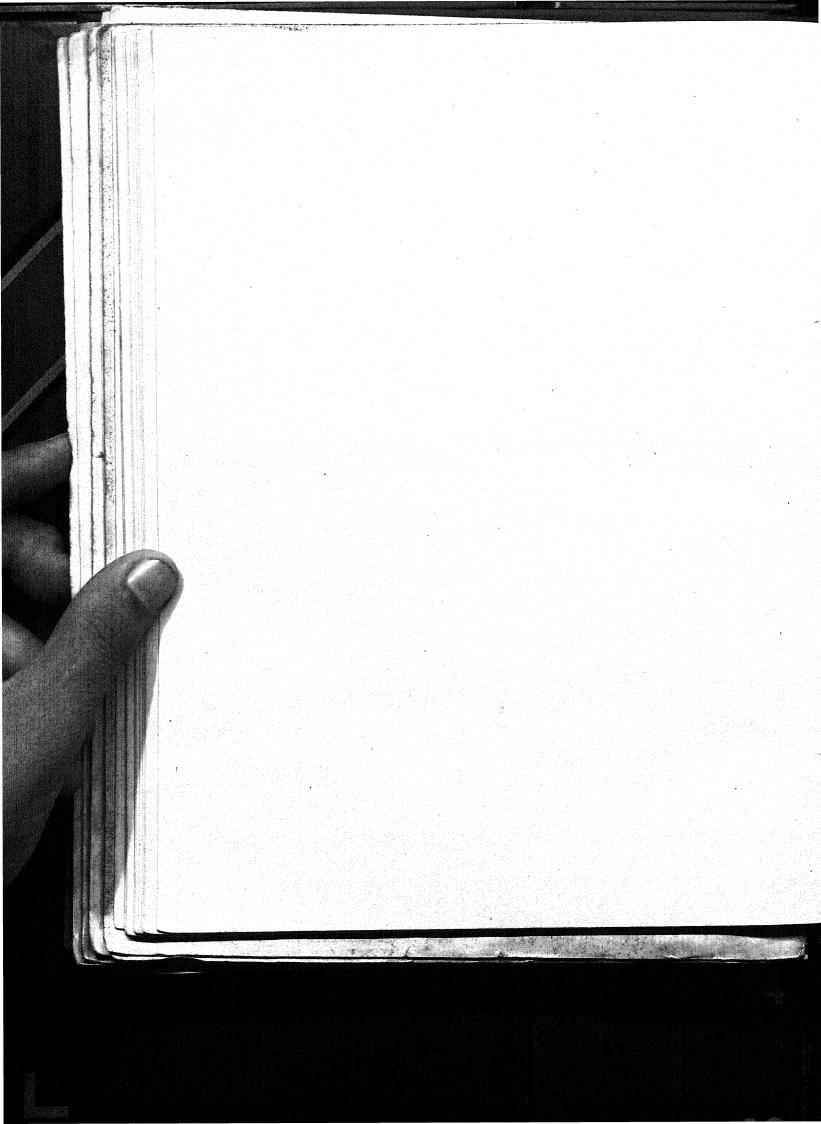


Fig. 2. Apparatus for extraction with chloroform



as the results of the analyses have been given in Table I. The results show that as growth proceeds and the polymerisation of the products of the photosynthetic activity results in the formation of cellulose,

etc. there is a gradual decrease in the percentage of inorganic constituents (16-10 per cent) and nitrogen (6-3 per cent) while resins show a fluctuation within a certain range (46—26 per cent) (Fig. 1).

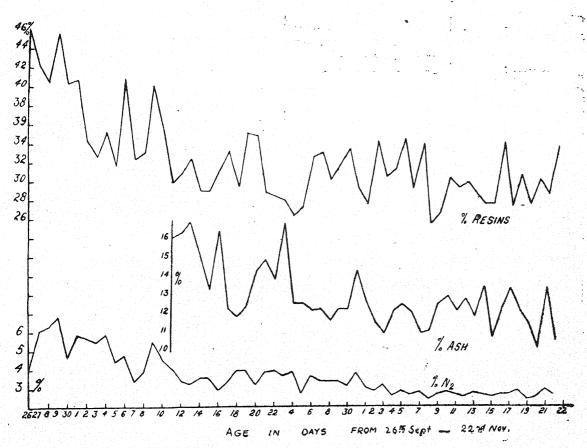


Fig. 1. Percentage of nitrogen, ash and resins of Cryptostegia grandiflora seedlings

## SUMMARY

A study of the metabolic changes of the inorganic constituents, nitrogen and resins of Cryptostegia grandiflora seedlings has been made in relation to their day to day growth till the plants are 62 days old. It was found that with polymerisation of the products of photosynthetic activity there was a gradual decrease in the percentages of nitrogen and inorganic constituents while resins fluctuated within

a certain range. An account of an apparatus designed to extract several samples at a time has also been given.

## ACKNOWLEDGEMENTS

Our thanks are due to Mr J. P. Anderson, Controller of Rubber, and Major H. R. Walden, Incharge, Wild Rubber Section, Department of Supply, for financial assistance for work on *Cryptostegia grandiflora*.

TABLE I

The growth observations and the results of analyses of Cryptostegia grandiflora seedlings

	Rate of	f growth		Analytical results					
Date when seedlings were pulled out	Age of seedlings in days	Average length of root of 10 seedlings in in.	Average length of stem of 10 seedlings in in.	Average No. of leaves of 10 seedlings	Fresh weight of 10 seedlings in gm.	Dry weight of 10 seedlings in gm.	Percentage of nitrogen	Percentage of ash	Percenta of resins
							No a	l nalysis was	done
23-9-43 · · · · · · · · · · · · · · · · · · ·	$\frac{1}{2}$	_	_	_	=	_		Ditto Ditto	
24-9-43	3		_		_			Ditto	
25-9-43	4		_			0.0824	4.08		46.34
26-9-43	5				0.38	0.0950	6.14		42.24
27-9-43	6	_			0.40	0.0690	6.32		40.83
28-9-43	7	0.2	1.3	2	0.39	0.0674	6.872		45.48
29-9-43	8	2·3 1·9	1.2	2	0.47	0.0656	4.688		40.45
30-9-43 · · · · · · · · · · · · · · · · · · ·	9	2.5	1.3	$\begin{array}{c c} 2 \\ 2 \end{array}$	0.50	0.0726	5.796	-	40 75
		2.6	1.3	2	0.54	0.0870	5.686	_	34.67
2-10-43	11	1.3	1.4	2	0.31	0.0774	5.524	=	32.81
3-10-43	12	1.5	1.7	2 2	0.63	0.0780	5.928	=	35.31
4-10-43	13	2.6	1.6	2	0.95	0.1202	4.436	_	31.84
5-10-43	14	2.3	1.5		0.81	0.1090	4.788	_	41.11
6-10-43	15	2.6	1.4	2	0.87	0.1010	3.296		32.59
7-10-43	16	2.1	1.5	3	1.15	0.1420	3.758		33.23
8-10-43	17	3.6	1.5	4	0.65	0.1306	5.548	-	40.20
9-10-43	18 19	2.3	1.5	4	0.94	0.2006	4.436		35.64
0-10-43 . ·	20	4.9	1.3	4	1.26	0.2530	3.972	16-14	30.06
	21	2.9	1.9	4	1.19	0.2376	3.344	16.31	30.90
12-10-43	21 22	3.7	1.9	6	1.63	0.3618	3.207	16.88	32.58
13-10-43	22 23	3.5	1	4	2.05	0.3394	3.532	-	29.24
4-10-43 .	23 24	3.5	* 2.2	5	1.16	0.2920	3.452	13.27	29.20
15-10-43	25	3.1	2.2	6	1.88	0.6268	2.819	16.38	31.3
16-10-43	26	3.2	$2.\overline{4}$	6	1.88	0.3546	3.169	12.97	33.49
17-10-43	27	3.2	2.5	6	1.92	0.4974	3.882	11.78	29.58
18-10-43 . •	28	4.1	2.4	6	2.85	0.4538	3.891	12.38	35.48
19-10-43	29	3.4	2.5	6	2.90	0.4500	3.232	14.34	35.1
20-10-43	30	3.6	2.7	5	2.97	0.4622	3.760	14.99	29.04
	31	3.5	2.5	6	3.10	0.4942	3.891	13.84	28.5
22-10-43 .	32	3.2	2.5	6	2.52	0.3476	3.561	16.90	28.3
23-10-43	33	3.1	2.9	6	2.88	0.4192	3.768	12.61	26.7
24-10-43	34	2.9	2.3	6	2.08	0.3104	2.559	12.59	27.6
25-10-43	35	3.6	2.0	6	2.32	0.4318	3.621	12.19	33.3
26-10-43	36	4.2	2.6	6	3.1	0.5800	3.282	12.33	33.4
27-10-43	37	$\hat{3}\cdot\tilde{7}$	2.5	6	3.0	0.6200	3.282	11.64	30.6
28-10-43	38	3.9	2.8	7	3.9	0.7200	3.312	12.33	32.2
29-10-43 30-10-43	39	3.8	2.5	6-	3.55	0.7006	3·141 3·784	12·16 14·32	33·9 29·8
31-10-43	40	4.8	2.8	8	4.37	0.9520	9.10#	17.02	
	41	5.2	2.6	8	4.39	0.8010	2.952	12.66	28.0
1-11-43	42	5.6	2.9	8	4.23	0.9526	2.845	11.53	34.8
2-11-43	43	5.2	2.9	7	3.22	0.6674	3.097	10.94	31.0
3-11-43	44	4.7	3.1	7	4.40	0.7846	2.506	12.07	31.8
4-11-43	45	4.8	3.0	8	3.40	0.6930	2.793	12.50	34.9
5-11-43	46	5-8	3.3	9	4.51	0.9458	2.630	12.05	29.8
6-11-43 . •	47	5.0	3.5	9	4.47	1.2744	2.660	10.88	34·5 26·0
7-11-43	48	6.0	3.5	9	5.17	1.2974	2.399	11.12	
8-11-43	49	5-6	3.9	10	6.37	1.4318	2.612	12.56	27.3
9-11-43 . · · · · · · · · · · · · · · · · · ·	50	5.5	3.7	10	5.6	1.2924	2.838	12.85	30.9

TABLE I (concld.)

The growth observations and the results of analyses of Cyptostegia grandiflora seedlings

	Rate of growth							Analytical results			
Date when seedlings were pulled out	Age of seedlings in days	Average length of root of 10 seedlings in in.	Average length of stem of 10 seedlings in in.	Average No. of leaves of 10 seedling	Fresh weight of 10 seedlings in gm.	Dry weight of 10 seedlings in gm.	Percentage of nitrogen	Percentage of ash	Percentage of resins		
11-11-43	51	2.6	3.3	9	5.36	0.9634	2.600	12.22	29.96		
12-11-43	$\frac{52}{52}$	5.6	3.7	10	7.47	1.4004	2.501	12.76	30.56		
13-11-43	53	5.6	3.9	10	8.27	1.5700	2.685	11.83	29.27		
14-11-43	54	4.7	3.1	9	4.05	0.8020	2.576	13.37	28.35		
15-11-43	55	5.5	2.2	11	10.28	1.9298	2.541	10.76	28.31		
16-11-43	56	6.2	3.1	11	6.9	1.2990	2.565	12.27	34.83		
177 11 49	57	6.0	4.0	10	11.1	2.3516	2.596	13.37	28.08		
18-11-43	58	6.5	3.9	10	6.2	1.8118	2.767	12.24	31.52		
19-11-43	59	5.5	3.7	10	6.9	1.5270	2.301	11.35	28.17		
20-11-43	60	4.8	3.5	9	7.15	1.4780	2.432	10.09	31.12		
21-11-43	61	4.3	3.8	10	5.9	1.5170	2.911	13.36	29.27		
22-11-43	62	4.2	4.1	9	6.78	1.5846	2.649	10.62	34.59		

N.B. The colour of the root was white, that of the stem and leaves was pink and green respectively after one week of germination.

## STUDIES ON THE BORON STATUS OF SOME BENGAL SOILS

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THOUGH the problem of boron nutrition of plants has been receiving increasing amount of attention in its various aspects in the hands of plant pathologists, soil scientists, biological chemists and fertilizer producing concerns in different countries of the world, particularly in America, practically nothing is known about the boron status of Indian soils and the influence of boron on Indian agriculture. In Europe and America, borax has already become a standard ingredient of fertilizers for certain crops and the practice is rapidly spreading with the progress of research in this field.

It is, therefore, a matter of first rate importance from the view point of practical agriculture to ascertain the deficiency or sufficiency of this element in Indian soils and to study the problems arising out of it. Once the deficiency is known to occur, the next steps would be to find out the localities affected, the extent of deficient areas, the various plants affected and the measures necessary to counteract the deficiency. The object of the present work is to throw some light on the boron status of some Bengal soils taken from different parts of the province.

## ANALYTICAL METHODS

Determination of boron in soil extracts. In the determination of boron in soil extracts, the quinalizarin colorimetric method of Berger and Truog [1939] was used all through this work. Preparation of colour standards, 98.5 per cent sulphuric acid solution by weight, quinalizarin solution and other reagents required in the determination were made exactly in the same way as was described by the above authors.

Determination of water-soluble boron of soils. After making numerous tests with water at different temperatures and by changing the mode of extraction, it was found by Berger and Truog [1939] that refluxing of the soil-water suspension for five minutes was the best procedure for extracting the water-soluble boron of the soil. The same authors [1940] showed that the results thus obtained gave good correlation with plant response to boron fertilization. De Turk and Olson [1941] showed that water-soluble boron is a fairly reliable measure of the available boron content of soils. This method is now widely used for the determination of available boron of soils and has been adopted in this work as well.

Determination of acid-soluble boron of soils. Richards et al. [1942] stated that digestion of a soil with a fairly strong acid would furnish a means for separating the maximum available boron from the soil. According to him, although tourmalline and other boro-silicate minerals will not be affected by strong acids, the boron in organic matter, in precipitated borates or boron in soil colloids should be readily liberated as boric acid by such a digestion and would therefore give a measure of the boronpresent in soil which, though not immediately available, would represent the portion available with difficulty. They used 85 per cent orthophosphoric acid as a digesting solution. Dilute acids have also been tried by some workers. Askew and associates [1937, 1938] extracted several New Zealand orchard soils with 0.05 hydrochloric acid and found 0.05 to 0.68 p.p.m. of boron in the extracts. Woodbridge [1940] found that water containing carbon dioxide extracted 0.09 to 0.33 p.p.m. of boron from several soils from British Columbia. Berger and Truog [1940] extracted eight soils with N/10 sulphuric acid both by shaking for 30 minutes and by refluxing for five minutes. From its analogy as an extractant for easily available soil nutrients such as phosphorus and potassium, N/2 acetic acid was used in this work. The extraction was done by refluxing for five minutes and the soilsolvent ratio was the same as in water extrac-

Determination of total boron of soils. In the determination of total boron in soil, the soil must be completely decomposed and fused as it contains highly resistant boro-silicate minerals. Alkaline fluxes, soidum carbonate, potassium carbonate, sodium acid phosphate may be used as a fusion agent. Berger and Truog [1939] found that fusion with anhydrous sodium carbonate followed by addition of sulphuric acid to bring the reaction of the final solution within a pH range of 5.52 to 6.00, hastens

the disintegration of the melt and leaves most of the sexquioxides and silica in insoluble form. The larger amount of sodium sulphate that is formed could be thrown down by the addition of alcohol upto 60 to 70 per cent by volume. The small amounts of non-volatile organic matter usually introduced with the alcohol may be destroyed by igniting the residue obtained after the final evaporation. McHargue and Hodgkiss [1941, 1942] using this procedure obtained satisfactory results for the total boron content of soils. Berger and Truog's [1939] method was used in this work for the determination of total boron of soils.

Water soluble boron by shaking method. It was pointed out by Berger and Truog [1939] that refluxing of the soil-water suspension (soil-water ratio 1:2) for five minutes was the best procedure for the determination of available boron of soils. Refluxing for periods either shorter or longer than 5 to 10 minutes extracted more boron. They were able to recover completely the soluble boron added to soils by this method. The addition of hot water followed by shaking for 30 minutes did not result in complete recovery. The effect of time of shaking on the available boron extracted was not, however, investigated by them. To see the effect of time on the amount of available boron extracted by the shaking method, the following procedure was adopted.

Twenty gm. samples of soils were weighed into shaking bottles and 100 c.c. of redistilled water added to each. The bottles were then shaken in a mechanical shaker for periods of 15 minutes, 30 minutes, 45 minutes and an hour. After the shaking, the soil was immediately filtered and boron was determined on an aliquot of the filtrate. Six soils representing different soil conditions and containing different amounts of water-soluble boron were selected for this work. The results are shown in Table I.

Table I Effect of time of shaking on the water-soluble boron in p.p.m.

Soil	15 min. shaking	30 min. shaking	45 min. shaking	I hour shaking	Refluxing for 5 min.
Dacca (No. 1)	0.87	1.00	0.87	0.63	0.63
Barisal (No. 4)	0.63	0.75	0.62	0.40	0.63
Berhampore (No. 10)	0.75	0.87	0.75	0.75	0.45
Badarkhali (No. 13)	1.00	1.12	1.00	0.87	2.00
Hijalbeel (No. 16)	0.75	0.87	0.75	0.62	0.40
Hawar area (No. 18)	0-63	0.75	0.62	0-45	1.12

It will be seen from Table I that the available boron extracted by shaking for 15 minutes is greater than that obtained by refluxing for five minutes in the soils having a low content of available boron, while in the two soils rich in available boron, shaking extracted less boron. It may mean that part of the boron present in the latter is present in a form which is not soluble in water at the ordinary temperature. It will be further seen that as the period of shaking becomes longer, the amount of boron extracted at first increases and then gradually falls off. The maximum amount of boron is extracted at the 30 minutes shaking period. This is true for all the six soils studied. It would appear that along with the solution of the soil boron, an opposing reaction by which boron is precipitated into insoluble compounds go on simultaneously and as time progresses the opposing reaction gets the upper hand. This is probably why Berger and Truog [ 1939 ] could not recover the whole of the added boron by shaking for 30 minutes with hot water while refluxing for five minutes effected complete recovery. This precipitation is probably counteracted if the extraction is done by boiling water and hence as the time of refluxing is lengthened, more and more boron (may

be of the more resistent type as well) come into solution. The five minutes refluxing procedure of Berger and Truog was finally adopted in this work for the determination of available boron.

#### RESULTS OBTAINED

The water-soluble boron, acid-soluble boron and total boron content of 26 samples under study are shown in Table II. pH organic carbon content and exchangeable calcium content of the soils are also shown in the same table. The soil samples analysed were collected from different parts of Bengal. They were selected for this investigation as representative of various types of soils found in different regions of the province. They covered both the acid and alkaline ranges of pH. In the case of soils obtained from the district farms and other experimental farms, whenever possible, samples were taken from both unmanured plots and from plots fertilized with different kinds of artificial fertilizers and organic manures. Some samples were taken from typical lowland areas where the soil remains submerged under water for four to five months and are specially adapted to the cultivation of 'boro' paddy.

Table II

Results obtained (boron expressed as p.p.m.)

Soil No.	Description	рН	Water- soluble boron	Acid- soluble boron	Total boron	Percentage of organic carbon	Percentag of ex- change- able calcium
		5.1	0.63	0.45	33	0.907	0.130
G1	Dacca Farm	5·2	1.37	1.12	55	0.887	0.080
G2	Bankura Farm, unmanured	5.2	0.87	0.75	100	0.731	0.132
G3 = -	Bankura Farm, manured	8.4	0.63	0.45	43	0.731	1.877
G4	Barisal Farm, manured	7.6	1.62	1.37	100	0.868	2.065
$G_5$	Barisal Farm, unmanured	8.2	1.12	0.63	100	0.936	0-064
G6	Midnapore Farm, manured	8.0	1.12	0.87	100	0.868	0.040
G7	Midnapore Farm, unmanured	8.2	1.12	0.87	90	0.960	1.527
G8	Rajshahi Farm, manured	8·2 8·2	0.87	0.62	100	0.956	0.882
G9	Rajshahi Farm, unmanured	7.0	0.45	0.62	100	0.936	0.066
G10	Berhampur Farm, unmanured	8.0	0.45	0.63	100	0.780	0.065
G11	Red Soil I, Vowal area	8.0	0.45	0.63	100	0.907	0.098
G12	Red Soil II, Vowal area		2.00	1.75	1.5 ° 3 ° 3 ° 3 ° 3 ° 3	0.751	0.064
G13	Badarkhali (Chittagong) 'Kosch soil' .	5.2	1.12	1.37		0.887	0.140
G14	Sutahata (Midnapore) Flood area .	8.0	0.45	0.75		1.180	0.088
G15	Hathhazaria I (Chittagong)	8.0	0.40	0.87		1.073	0.177
G16	Hijalbeel (Murshidabad) Lowland	8.0		0.75		0.468	0.370
G17	Tista Silt (Rangpur)	7.6	0.45	0.87		1.580	0.168
G18	Hawar area I (Mymensing)	5.4	1·12 0·50	0.45		0.919	0.140
G19	Hawar area II (Mymensing)	6.4		0.43	••	1.082	0.263
G20	Daulatpur Farm (Khulna)	8.0	1.12	0.87		0.539	0.177
G21	Jessore Farm	6.4	1.12	0.50		0.406	0.066
G22	Bankura, Highland	5.2	0.75	0.50		0.410	0.564
G23	Suri Farm	8.0	0.87	The second of th		0.662	0.045
G24	Rangamati (Chittagong)	4.4	0.50	0.45	•	0.254	0.043
G25	Hathhazari II (Chittagong)	4.4	0.75	0.63	••	1.034	0.241
G26	Paralbari (Midnapore) Flood area	5.0	1.37	1.12	•	1.034	0.741

DISCUSSION OF RESULTS

Effect of pH on availability of boron. It will be seen from Table II that the available boron content of the 26 soils under study ranges from 0.4 p.p.m. to 2.0 p.p.m. The average comes to 0.9 p.p.m. pH of the soils ranges from 4.4 to 8.4. In the 12 acid soils the available boron comes, on an average, to 0.95 p.p.m., whereas, in the 14 soils having their pH above 7.0 and extending up to 8.2, the average of available boron comes to 0.86 p.p.m. There is also considerable variation in the available boron of soils having the same or nearly the same pH. For example, soil Nos. 6, 7, 8, 9, 11, 12, 14, 15, 16, 20 and 23 having their pH at 8.0 or in the neighbourhood, contain all amounts ranging from 0.40 to 1.12. These results show that the amount of available boron present in a soil is determined by factors other than its pH. The nature of the parent material, the extent of weathering and leaching, the system of cropping and the chemical composition of the soils may be assumed to play a large part in determining the available boron content of a particular soil. The results obtained by previous workers are of somewhat conflicting nature. Haas [ 1937 ] stated that a deficiency of boron is most likely to occur on alkaline or overlimed soils while Brandenburg [1932] and Cook [1940] reported that the heart-rot of sugar beets was common on acid as well as on alkaline soils. Cook and Millar [ 1939 ] observed that there were so many cases of deficiency on acid soils and non-deficiency on alkaline soils that other factors than pH value were of greater importance. According to them supply of calcium and organic matter, and soil texture were most important in affecting boron availability. Purvis and Hanna [1939] reported that majority of boron deficient areas in America occurred on acid soils.

Effect of organic matter and exchangeable calcium on boron availability. Cook and Millar [1939] believed that permeability of the sub-soil horizon, supply of calcium and organic matter and soil moisture were the most important soil factors affecting boron availability. They showed that there was a correlation between the appearance of heart-rot in sugar beets and active calcium content of the soils. Even when the effect of pH was minimized by placing the soils into acid and alkaline groups, there was evidence to show that the soils on which heartrot of sugar-beets occurred were higher in active calcium content then were the soils on which heartrot did not occur. Purvis [1939] found that tolerance of soils to application of borax was correlated fairly well with the organic matter content and exchange capacity. Less injury was produced by berax in soils having a high organic carbon content and high exchange capacity than in a soil having a lower carbon content and exchange capacity. The results obtained in this work, however, show that there is no apparent relation between the availability of boron in the soils and their organic carbon or exchangeable calcium content.

Acid-soluble boron. The results show that in the majority of soils, the amount of boron dissolved by N/2 acetic acid is less than water-soluble boron. Out of the 26 soils, only in six soils the acid-soluble boron is higher than the water-soluble boron. All these soils are alkaline in reaction, having a pH of 8.0 in four cases. But as there are other alkaline soils in this series which have shown a decrease, no generalization is possible on the basis of pH. The ratio of water-soluble boron to acid-soluble boron falls within a very narrow limit; in most of the soils the ratio varies from 1.1 to 1.4, which shows that in the majority of soils acetic acid extraction method will give a relative measure of the available boron content of soils.

Total boron. Total boron was determined accurately only in a few samples; in most of the remaining soils the quantity was much above 100 p.p.m. and could not be determined accurately as the colour developed was too blue to be compared with the standards. Much of this total boron is present as particles of resistant rocks and minerals residual from the decomposition of the parent material. The most commonly identifiable boron mineral in soils is tourmaline which is a complex aluminium boro-silicate of iron, magnesium or other bases and which contain 3.1 per cent of boron. The boron present in this form is highly unavailable to plants. The results obtained by previous workers show that many soils contain high quantities of total boron though the available portion is usually very small. Bertrand and Silverstein [1939] analysed 24 soils from Europe and Africa for their total boron content and found it to vary from 7 to 50 p.p.m., 75 per cent of them between 10 to 30 p.p.m. In 10 soil samples, Luchetti [1938] found the total boron to range from 20 to 100 p.p.m. Rogers and associates by spectroscopic analysis, estimated as much as 100 to 500 p.p.m. of boron in certain soils from Florida.

Distribution of available boron by areas. In the absence of any well defined classification or grouping of the Bengal soils, it is difficult to say with any amount of precision whether the deficiency or non-deficiency of boron is connected with any particular soil type of the province. It may, however, be observed that the soils taken from the Chittagong district are low in their available boron content. The soil Nos. 15, 24 and 25 taken from that area contain 0.45, 0.50 and 0.75 p.p.m. of available boron respectively. Soil No. 13 (Badarkhali) taken from the coastal tract of Chittagong is the only exception. This soil contains the highest amount of available boron, namely, 2 p.p.m. This soil which goes by the name of 'Kosch' soil, was

included in this series because of its high infertility which cannot be ascribed to any of the known causes. The result obtained here is interesting because the soil is found to contain a very high amount of available boron which may fall within the border line of toxicity and thus be partly responsible for the unproductivity of the soil. The soils from the district of Midnapore which are subjected to occasional flooding by sea water reveal great similarity in their available boron content, the quantity being much higher than those of the Chittagong area. Thus soil Nos. 6, 7, 14 and 26 from this area contain 1·12, 1·12, 1·12 and 1·37 p.p.m. of available boron respectively. This high figure must be due to inundation of the area by sea water. It has been shown by Moberg and Harding [1933] that the concentration of boron in sea water is about 4.5 p.p.m. Richards et al. [ 1942 ] have stated that irrigation water may add boron to the soil of the cultivated areas. From a comparison of water soluble boron of a number of irrigated and non-irrigated soils, they found that the irrigated soils contained 2.84 p.p.m. of water soluble boron on an average as compared with an average of 0.87 p.p.m. in the non-irrigated soils. According to them much of the boron added by irrigation water is retained by the soil in a readily soluble form, thereby considerably increasing the water-soluble boron content. The soils from Barisal, Khulna and Jessore (Nos. 4, 5, 20 and 21) forming part of the saline tracts of the Sundarbans also show a similarly high figure for available boron (average 1·12 p.p.m.). The three uncultivated soils from Dacca, Hijalbeel and Tista (Nos. 1, 16 and 17) are fairly low in available boron. Similar is the case with the red soils from the Vowal area. The average boron content of the soils taken from the interior, namely, Rajshahi, Bankura, Berhampore and Suri shows an average which is nearly equal to the general average of the available boron in the 26 soils studied. There does not seem to be any consistent difference between boron availability of manured and unmanured soils of the same locality.

The results obtained here do not, however, allow any inference to be drawn as to whether the concentration of boron usually present in Bengal soils falls within the deficiency or toxic concentration of the element. Such an inference is only possible when extensive field trials are made on different types of soils and with different kinds of crops and the laboratory results are calibrated. From the results obtained by various workers in other countries, it appears that the concentration of boron below which deficiency symptoms will appear, lie within a range of 0.25 to 0.4 p.p.m. and that boron will begin to show its toxic effect as it approaches a concentration of 2 p.p.m. From this point of view, the Bengal soils studied do not seem to indicate either

excessive or deficient concentration of available boron.

Boron present in organic combination. Boron is added to the soil with every addition of vegetable and animal matter and with decay and decomposition of the organic residues, it will be liberated in inorganic forms. As the decomposition of the organic matter is constantly going on in the soil through the agency of micro-organisms, the soil organic matter may be looked upon as a reserve for the available boron. It was thought that determination of the organic boron content of soils might have useful information to a study of the boron status of soils. No method has yet been devised for estimating the boron that is present in soils in organic combination

It is known that when soil material is ignited, organic matter is destroyed and therefore the mineral nutrients are converted into inorganic forms. The increase in the soluble constituents of a soil caused by ignition may therefore be assumed to be derived from the mineralization of the soil organic matter. If the extraction is done under identical conditions, the increase would represent the portion which has come from the organic matter by its destruction or in other words the increase will represent the organic form of the element. This principle has been utilised in determining organic boron of the soils.

Twenty gm. of soils were weighed in a porcelain basin and the basin was placed inside an electric muffle. The muffle was adjusted at about 600°C and the ignition was carried out for an hour. The sample was then cooled, transferred to the distilling flask and boiled under a reflux condenser with 40 c.c. of distilled water for about five minutes. Boron was then determined on an aliquot of the extract. From this was subtracted the amount of boron extracted from the unignited soil under identical conditions of extractions. The difference was taken to represent the organic boron of the soil. The results are shown in Table III.

Table III

Organic boron in p.p.m.

Soil Water-soluble No. boron of fresh soil		Water-soluble boron of ignited soil	Increase due to ignition	
GI	0.63	1.87	1.24	
G2	1.37	1.62	0.25	
G3	0.87	0.87	0.00	
G4	0.63	0.63	0.00	
G5	1.62	2.37	0.75	
G5 G6	1.12	1.87	0.75	
G7	$1.1\overline{2}$	1.62	0.50	
	$\hat{1}\cdot\hat{1}\hat{2}$	1.75	0.63	
G8 G9	0.87	1.37	0.50	

Table III (concld.)
Organic boron in p.p.m.

Soil No.	Water-soluble boron of fresh soil	Water-osluble boron of ignited soil	Increase due to ignition
G10	0.45	0.75	0.30
G11	0.45	0.75	0.30
G12	0.75	0.87	0.12
G13	2.00	2.75	0.75
G14	1.12	2.25	1.13
G15	0.45	2.12	1.67
G16	0.40	2.12	1.72
G17	0.45	2.12	1.67
G18	1.12	2.00	0.88
G19	0.50	2.12	1.62
G20	1.12	2.12	1.00
G21	1.12	1.12	0.00
G22	0.75	2.12	1.37
G23	0.87	2.12	1.25
G24	0:50	1.87	1.37
G25	0.75	2.63	1.87
G26	1.37	1.37	0.00

It will be seen from Table III that in most of the soils ignition has increased the amount of water-soluble boron. In some cases the increase is as high as 1.87 p.p.m. In four soils there is no increase, showing that there is little boron present in organic combination whereas in half a dozen soils the increase is small as compared with others.

#### SUMMARY

Twentysix soil samples taken from different parts of Bengal were analysed for their available boron content by the quinalizarin colorimetric method of Berger and Truog. The available boron content of these soils ranges from 0.4 p.p.m. to 2.00 p.p.m., the average being 0.9 p.p.m. The pH of the soils has little effect on the availability of soil boron. In the 12 acid soils the average comes to 0.95 p.p.m. whereas in the 14 soils having their pH above 7 the average is 0.86 p.p.m. of boron.

In most of the soils boron soluble in N/2 acetic acid is less than the amount in water-soluble form. The ratio of water-soluble boron to acid-soluble boron falls within the range of 1·1 to 1·4 in most cases.

There is no apparent relation between the availability of boron and the organic matter or exchangeable calcium content of the soils studied.

The soils taken from the district of Chittagong are generally low in available boron whereas the soil taken from the district of Midnapore which are subjected to occasional flooding by sea water contain a much higher percentage of boron. The soils from the saline tracts of Sundarbans also show a similarly high figure.

The amount of bo on extracted by shaking with water is less than that obtained by refluxing for five

minutes in the soils having smaller amounts of available boron while shaking extracted less boron from the soils which are rich in available boron. The amount of boron extracted by water at first increases with the increase in the period of shaking reaching a maximum at the 30 minutes shaking period after which it falls off slowly.

Ignition of the soil in most cases increased the amount of water-soluble boron, sometimes by as much as 1.87 p.p.m. The difference between the water-soluble boron of the ignited and the fresh soil has been assumed to represent the boron present in the soil in organic combination.

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#### SOME OBSERVATIONS ON THE GENETICS OF LINSEED

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Linseed ranks second in importance among the oilseeds of the United Provinces, the first place being held by Brassicae. The area under linseed in the United Provinces is about 900,000 acres, which is roughly 2½ per cent of the total cropped area of the province. Relative to the linseed acreage in India, the crop occupies about 22 per cent of the area in the United Provinces which comes next only to the Central Provinces and Berar which claim 28 per cent of the area. But in the matter of production, the United Provinces ranks first, contributing 30.9 per cent of the total produce, followed by Central Provinces and Berar which contribute 17.9 per cent.\*

Breeding work on linseed was started at the Botanical Research Farm, Cawnpore, as early as 1924-25 when a collection of varieties of Indian linseeds and foreign flax varieties was made. In subsequent years crosses were made between the indigenous and exotic varieties, with a view (1) to combine rust

resistance of the flax varieties with the higher yield and oil content, earliness and bigger seed size of the indigenous types and also (2) to isolate flax like hybrids suitable for conditions obtaining in the United Provinces. One of the crosses made with the latter object has given a number of hybrid forms which while retaining, and some of them exceeding, straw length of the flax parent possess a suitable duration and adaptation to local conditions. The exotic flax types were never a success due to the poor germination, long duration and defective seed setting. The extracted hybrids are free from these shortcomings. Two of these, viz. Fx3 and Fx6, are now available for multiplication.

The above cross also provided an opportunity for the study of inheritance of colour character of the flower. The parents were English White and C. P. White; their characters are recorded in Table I.

Table I

The characters of C. P. White and English White

Parents	Flowers	Petal	Anther	Seed
C. P. White	Large, open	White with pink tinge and violet streaks		Dull white
English White .	Medium sized, not fully open	White with very faint bluish tinge	Salmon	Brown

The types were crossed in 1928-29, and later again crossed in 1938-39 to confirm the observations made earlier.

 $F_1$  Generation. The  $F_1$  plants had, unlike the parents, blue corolla, and the anthers were light blue and the seeds brown in colour.

 $F_2$  Generation. The  $F_2$  generation was studied for the segregation of corolla colour. Four main phenotypic classes were observed, viz. two parental classes, one class like the  $F_1$  and a new white-petalled class. They have been termed C P W (to represent the corolla colour of C. P. White), P W (to represent the corolla colour of English White), Blue (the P class) and P W (the new white-petalled class). The frequencies were as follows (Table II).

The values of  $X^2$  in the two cases are, respectively, 1.91 and 1.87. The corresponding values of P are 0.603 and 0.604 respectively, which shows that the fit is good in both the cases.

 $F_3$  Generation. The data obtained in  $F_3$  were in close agreement with the expected segregations of the hybrids and are not presented here. The frequencies given by the segregating  $F_3$  families were recorded and the fit in every case was very close.

The behaviour of corolla colour in inheritance is similar to that recorded by Shaw, Khan and Alam [1931]. In a cross between two linseed types, I and 12, possessing corolla colour similar to that of C. P. White, and English White respectively, they secured in F<sub>2</sub> a dihybrid segregation. However, they observed a deficiency in the double recessive phenotype which they ascribed to some lethal combination of two factors. It is quite possible that the size

<sup>\*</sup> See Report on Marketing of Linseed in India Published by the Manager of Publications, Delhi

# Table II The frequencies of phenotypic classes in $F_2$ generation

		Total			
	Blue	CPW	EW	W	10tai
Observed	1504 1486•1	471 495•4	493 495•4	174 165•1	$\frac{2642}{2642}$
C. P. White x English White  Observed	1443 1466	489 489	497 489	178 163	2607 2607

of F<sub>2</sub> population studied by them, viz. 342 plants, was not large enough to give the expected strength of the double recessive phenotype, and that with a larger population no such deficiency might have been observed. In the above cross between C. P. White and English White, no such deficiency was observed. However, the inheritance can be explained according to the theory applied by Shaw, Alam and Khan. Employing the genic symbols used by them we can represent the constitution of the corolla of the two parents as follows:—

C. P. White—BB CC dd EE FF. Eng. White—bb CC DD EE FF.

B is a factor which acts with C to produce pink colour in the petal.

C is a factor for colour in the petals and acts

with B to produce pink.

D is a factor which modifies pink to lilac.
In the absence of B and the presence of E, it causes a faint tinge of blue.

E is a factor which intensifies colour in the petal. F is a factor which dilutes pink colour in the

petal.

The F<sub>1</sub> of the above cross had blue petals and had the constitution BbCCDdEEFF. The F<sub>2</sub> had the four phenotypes in the proportion 9:3:3:1 as follows:

				1 bCdEF
			3 bCDEF	
	O BCDEF	3 BCdEF		
				William mith mo
		1111 14 - Miles (* 1)	White like Eng	White with no
	des III a W	White like C. P.	White like Eng.	White with no
1	dne like F.	White like C. P.	White like Eng.	
J	slue like F <sub>1</sub>	White like C. P.	White like Eng.	
J	Slue like F <sub>1</sub>	White like C. P.	White like Eng.	White with no blue tinge
J	slue like F <sub>1</sub>	White like C. P. White	White like Eng. White	
1	Slue like 🗜	White like C. P. White	White like Eng. White	
J	slue like F <sub>1</sub>	White like C. P. White	White like Eng. White	
]	Slue like F <sub>1</sub>	White like C. P. White	White like Eng. White	
]	slue like ₹ı	White like C. P. White	White like Eng. White	
J	slue like F <sub>1</sub>	White like C. P. White	White like Eng. White	

#### ANTHER COLOUR

Among the numerous hybrid families raised in different years segregations for anther colour, seed colour, height, duration and habit were observed and were recorded in some cases. Thus in 1940-41 counts were made in 49 segregating families for an-

ther colour for the phenotypes 'light blue' and 'salmon' colours. The segregation was mono-genic, giving in every case the ratio 3: 1 with the usual variation. The totals for the 49 families are:

	Light blue	Salmon
Observed · · · · · · Expected · · · · · · · · · · · · · · · · · · ·	2291 2296·5 3	771 765•5 1

The fit is good.

#### SEED COLOUR

Counts of brown and fawn seeded segregates in 19 families gave the following frequencies:

		Brown	Fawn
Observed		 771	249
Expected Ratio .		$\begin{matrix} 765 \\ 3 \end{matrix}$	255 1

The difference in seed colour thus appears to be due to a single gene, a fact which agrees with the findings of Ali Mohammad and Khan [1941] who ascribe the brown seed colour to the interaction of two factors G and M and fawn colour to the operation of M alone and absence of G.

#### DISCUSSION

The similarity between the results presented above and those described by Shaw, Khan and Alam [1931] and by Ali Mohammad and Khan [1941] is significant. This similarity would only mean that although the material which the different workers used represented widely different agricultural and commercial varieties of this crop, it possessed similar, if not

identical, genes for colour of petals, anthers and seeds. It would appear, therefore, that the genic constitution of linseed is comparatively simple in spite of the great variability shown by this crop. Unfortunately the records on the genetics of linseed are meagre and whether the genic constitution is more complex than what appears from recorded work, is a question which must be left to the future.

#### SUMMARY

The inheritance of petal colour in a cross between two pure lines of linseed was studied and was found to be governed by five genes, the parents differing from each other in two genes only. The results agree with those obtained by Shaw, Khan and Alam, viz. a dihybrid segregation into four phenotypes in the ratio 9:3:3:1. The double recessive phenotype, however, did not show any deficiency in number as that observed by the other workers.

The inheritance of anther and seed colour was also

studied and was found to be monogenic.

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#### METHODS OF COMPUTING PEST INCIDENCE

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(With one text-figure)

As expected an insect pest generally attracts the serious attention of an economic entomologist only when at least once its attack assumes such devastating severity that the problem of its immediate control overshadows all other considerations. rally therefore, under such conditions enough thought cannot be devoted to such matters of scientific accuracy as those of methods employed for estimating intensity of attack or measuring pest incidence, etc. But when these so to say emergency methods are recorded in print they naturally are followed up by subsequent workers who overlook the emergency character of those methods. Due to this circumstantial sequence for which nobody can be said to be really responsible, we find today in the literature on economic entomology that a large number of defective methods of computing and comparing pest incidence are being continued by worker after worker. The aim of the present article is to invite the attention of entomologists to some typical examples showing the nature of flaws existing in the present day common methods. An effort has been also made to offer an easy correction for the main flaw. Of course, it is by no means intended to belittle the works of previous investigators; the apalogy for the critical analysis presented herein lies in the fact that as result of the labours of the same previous workers now economic entomology has reached that proud stage when it requires all the accuracy of exact sciences and when at least glaring flaws in its technique must be examined and corrected,

COMMON METHODS OF COMPUTING INCIDENCE

As will be clear from the actual references some of which are cited later on, the incidence of both pests and parasites is generally recorded in the form of 'Percentage Values'. The pest is recorded as percentage of the material liable to be damaged; for example in the case of cotton bollworms it is a common record that bollworm incidence was say 50 per cent, meaning thereby that 50 bollworms were found per 100 buds and bolls examined or 50 per cent of the bolls and buds examined had bollworms. Again in the case of sugarcane borers we often come across statements like 'top-borer incidence was 10 per cent', 'stemborer incidence 15 per cent' and so on. Similarly parasite incidence is recorded as percentage of the host insect examined.

Some authors have made slight deviations from the above general practice; some of these deviations afford comparatively a little better method, some mean essentially the same expressed in different terms and there are some others which at first thought appear to constitute improvements but are actually much worse. For example, some workers on cotton bollworms have expressed incidence as 'number of bollworms per 25 plants' which is the same thing as percentage incidence with respect to the number of plants instead of percentage incidence with respect to the number of buds and bolls. As will be shown later this deviation is a little for the better. Workers on leaf-miners and leaf-suckers like aphids and white-flies express incidence as 'number of insects per

leaf' which is the same things as percentage incidence with respect to the number of leaves. Other workers on the same pests express the incidence as 'number of insects per unit area of leaf surface'. As will be shown later this is an example of deviation which is apparently more scientific than incidence expressed as number per leaf but is actually much worse.

#### THE CHIEF FLAW AND ITS CORRECTION

The chief flaw in all the above methods is that they ignore the fundamental axiom that the scale used for measurements must be constant if the measurement is to be reliable. When the incidence of a pest is recorded as percentage of the material liable to be damaged, the quantity of the damageable material becomes the scale against which the pest population is measured. Thus the number of buds and bolls becomes the scale in the case of bollworms, the number of canes in the case of cane-borers, the number of leaves or area of leaf-surface in the case of leafminers, aphids, whiteflies, etc. Similarly when we express the incidence of a parasite as percentage of the host parasitized, the number of the host insect becomes the scale against which the parasite population is measured. Now these scales are obviously not constant. In nature both the quantity of damageable plant material in the case of pests as well as the quantity of parasitizable insect host material, in the case of parasites practically vary from zero to infinity in the same season. The plant material, i.e. crop is absent, then it is sown, and it germinates, then it gradually grows in size and quantity and is finally harvested away; similarly the insect host material, i.e. pest is at first practically absent, then it makes its appearance, and its number gradually increases and then decreases again. This is an illustration of only seasonal variation in these scales; there are variations due to several other factors as will be clear shortly. Thus the scales with which we-measure the incidence of pests and parasites are subject to such wide fluctuations that the measurements, i.e. 'percentage incidence 'values, cannot be expected to be reliable or comparable at all. Let us take a concrete example of bollworm percentage Suppose at the time of first observation incidence. the field contains 100,000 buds and bolls and there are 10,000 bollworms distributed in the field, then if we conduct sampling in the usual way we will get 10 bollworms per 100 buds and bolls examined, i.e. we will record 10 per cent incidence. Further suppose that when the same field is examined after some time for second observation the number of buds and bolls in the field has increased to 200,000, but the number of bollworms has remained unchanged at 10,000, then in the second observation the sampling will give only 5 bollworms per 100 buds and bolls examined, i.e. we will record only 5

per cent incidence. The comparison of these two observations, i.e. 10 per cent and 5 per cent incidence will ordinarily lead us to conclude that bollworm activity has decreased in the field due to some reason although obviously it has not been actually the case. These observations will lead us to an erroneous conclusion simply because the scale used in measuring the incidence has not been constant, i.e. the number of buds and bolls has changed. That such erroneous conclusions are actually arrived at in practice due to misleading nature of 'percentage incidence' is amply proved by the following actual observations in the field: While studying the population fluctuation of spotted bollworm of cotton, we have been recording the total number of bollworms per acre, the total number of buds and bolls per acre, and also the percentage incidence of bollworms with respect to the number of buds and bolls. All the three sets of data for one complete season are graphed in Fig. 1. From this figure it will be clear how the percentage incidence often gives absolutely wrong idea. For example, starting from e in Fig. 1, it will be seen that the number of bollworms rises or at least remains constant up to the point f, but since the number of buds and bolls has considerably risen from e to f the percentage incidence shows a steep fall. Again after the point n, as the number of buds and bolls is rapidly decreasing the percentage incidence is showing rapid rise though actually there is little rise in the actual number of bollworms. In fact it is needless to point out the other discrepancies which exist: where the trend of change is indicated rightly the rate of change is hardly correct. These are the difficulties met with even when we compare the data from week to weak only; the flaw becomes all the more glaring when we compare the data from year to year. Thus when we compare our own data on the basis of 'percentage incidence' of spotted bollworm with respect to buds and bolls for the three seasons (i. e. 1941-42, 1942-43, 1943-44) it appears beyond doubt that infestation was entirely different from season to season, the highest percentage incidence in the three successive seasons being 10 per cent, 5 per cent and 21 per cent respectively; but when we compare the population per acre for the same three seasons we find that the population was almost the same, the highest level reached in the different seasons being 23,000, 20,000, and 22,000 respectively. Thus the percentage incidence data alone would have erroneously indicated undue apprehension in 1943-44 and an unduly false sense of security in 1942-43, and would have set the entomologists thinking about the cause of these differences.

Probably recording of 'percentage incidence' has come in vogue under the impression that the quantity of the host material remains so huge as compared with that of the parasite that the former can be

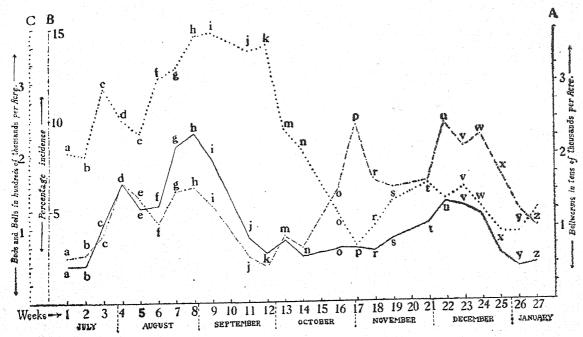


Fig. 1—The weekly fluctuation in the population of the spotted bollworm of cotton at Delhi during 1941-42.

- A. The weekly fluctuation in the actual population per acre, the numbers 1, 2, 3 on its scale representing tens of thousands;
- B. The weekly percentage incidence of bollworms with respect to buds and bolls, the numbers on its scale representing percentage figures;
- C. The weekly population of buds and bolls per acre, the numbers on its scale representing hundreds of thousands.

taken as constant like the level of the sea. The falacy of this impression will be clear from the theory of biological control in which effort is made to reduce the population of the host with the help of parasite. But surprisingly even in work on biological control 'percentage incidence' method is being used by many authors. Obviously the quantity of the host cannot be taken to be constant otherwise the experiment will be against the presumption. If the host material is constant it cannot be controlled and if it can be controlled it cannot be constant.

#### DEFECTIVE METHODS ILLUSTRATED

We are giving below a few typical illustrations from entomological literature showing the magnitude of error likely to creep in the results in case the fundamental axiom pointed out above is ignored. These illustrations will also show that the arguments advanced against the common method of computing percentage incidence are also applicable with greater or lesser force to the various modifications of that common method mentioned before.

The letters a to z represent the points on the three graphs corresponding to the numbers along the abscissa which represent serial numbers of the weeks, i.e. 1. week ending on 12th, 2. on 19th, 3. on 26th July, 4. on 2nd, 5. on 9th, 6. on 16th, 7. on 23rd, 8. on 30th August, 9. on 6th, 10. on 13th, 11. on 20th, 12. on 27th September, 13. on 4th, 14. on 11th, 15. on 18th, 16. on 25th October and so on.

1. Taking examples from Indian literature only the extensive work on pink bollworm right from the early papers of Fletcher and Misra [1921], Willcocks [1921], Ballard [1922], up to most recent papers and reports, is based on 'percentage incidence' only. For instance in a paper entitled 'Incidence of Platyedra gossypiella in relation to climate (1926-31) by Haroon Khan [1938] both the regional variation as well as the seasonal variation have been studied with the help of 'percentage incidence' only and on the basis of this study Punjab has been divided into four zones wherein 'percentage incidence' has been shown to vary between different limits. Now if the same zones are compared as regards number of bolls per acre there is obviously some difference between these four zones and this difference also must have resulted into some difference in the values of 'percentage incidence 'even if the population of pink bollworms per acre were to be the same in all the zones. If the difference in these four zones as regards number of bolls per acre is in the same direction as the differ. ence in population of bollworms per acre, then the

calculation of 'percentage incidence' as reported by the author must have masked the actual difference in bollworm activity in these zones. Therefore the differences shown by the author's data on percentage incidence must be regarded as underestimates. On the other hand, if the differences in bolls per acre and bollworms per acre are in opposite direction then the differences shown by this author's data must be regarded as overestimates. It will be instructive to consider in this connection that the yield of cotton per acre probably decreases as we travel from western part of the Punjab to eastern Punjab and if this decrease in yield is due to decreasing number of bolls per acre from west to east then, even if the bollworm population be uniformly distributed in the province, we should still find mathematically a progressive increase in percentage incidence from west to east as reported by the author, but this progressive increase will be due to mathematical principle and not due to any ecological law so far as bollworm distribution is concerned. Of course we do not mean to contend that the whole of the difference in percentage infestation between different zones is due to difference in boll production but the data given in the paper under reference do not show as to how much the difference in boll production is responsible for This factor difference in 'percentage incidence'. of boll production would not have interfered with the estimate if the data would have been given in terms

of population per acre. 2. Again in a more recent paper by Afzal Husain and Haroon Khan [1940], the Indian and American varieties of cotton have been compared with respect to pink bollworm infestation and here also the same flaw exists since only 'percentage incidence' has been taken as the criterion. Now if the desi and American varieties are compared as regards the number of bolls per acre there is bound to be some difference and this difference will itself create some difference in the values of 'percentage incidence' even if the bollworm number per acre be the same in desi and American varieties. Probably the number of bolls per acre may be less in the case of American than in the case of desi varieties (although the yield per acre may be more in American due to larger size of individual bolls), and the higher 'percentage incidence' in American cotton may be partly due to this fact. Our contention holds good even if the case is just the reverse, i.e. even if the number of bolls per acre may be more in American than in desi varieties in which case the difference shown by the authors between these varieties should be regarded as underestimates.

3. The monumental work on spotted bollworms carried out from 1923-31 by Deshpande and Nadkarny [1936] is based on 'number of bollworms per 25 plants' which as explained before, is the same thing as 'percentage incidence' with respect to plants.

This criterion contains the flaw already discussed by us although to a lesser degree. Such data are comparable from week to week if they are taken from the same field because the number of plants does not change much from week to week in the same field but such data are not comparable from year to year when crop changes and the number of plants also changes. Even if population per acre remains the same during different seasons, the season in which the germination remains comparatively poorer gives larger number of bollworms per plant or per 25 plants because the same population distributes itself on a lesser number of plants; similarly better germination produces the reverse effect. Our own data referred to above show that although the highest level of population per acre reached during the three seasons (1941-42, 1942-43, 1943-44) varied only as 23,000, 20,000 and 22,000, yet due to differences in population density of plants per acre during different seasons, the maximum value of number of bollworms per 100 plants fluctuated during the same period as 280, 110 and 384 respectively. Thus the work of Deshpande and Nadkarny contains detailed weekly data on number of bollworms per 25 plants for six consecutive seasons (1925-31) and the maximum number of bollworms per 25 plants recorded for these different seasons are 87, 144, 78, 130, 204 and 220. These data without being converted into population per acre cannot give any comparative idea as to the extent to which the different seasons were favourable or unfavourable for bollworm multiplication because the number of plants was not uniform during all the six seasons.

4. Very recently Trehan [1944] has studied incidence of whitefly of cotton by calculating number of immature stages of the whitefly per square inch of leaf area. This criterion is even worse than 'percentage incidence' based on the 'number of leaves' as leaf area is more variable than leaf number. Thus, for example, suppose in a field there are 1,000 leaves and suppose the average area per leaf is 4 sq. in. in the young crop of cotton, the total leaf-area of the field would be 4,000 sq. in. Now the whole population (say 20,000) of immature stages of whitefly will be distributed over an area of 4,000 sq. in. and a sampling will give five insects per sq. in. or twenty insects per leaf. Now suppose at the time of the next observation the insect population has remained practically unchanged at 20,000 but the number of leaves has increased from 1,000 to 2,000 and also the size of leaf has increased from 4 sq. in. to 8 sq. in. Now the total leaf-area of the field will be 16,000 sq. in, over which the population of 20,000 insects will be distributed. Therefore the sampling will show 20,000/16,000, i.e. 1.25 insects per sq. in. of leaf-area or 20,000/2,000, i.e. ten insects per leaf. Thus although the population

of the insect in the field has remained unchanged the number per leaf will show a decrease to one-half. i.e. from 20 to 10 whereas the number per sq. in. will show a decrease to one-fourth, i.e. from 5 to 1.25. Thus neither the number per leaf nor number per unit area of leaf surface can give the correct picture but the latter is more incorrect than even the former although at first thought the latter appears to be more scientific. On the basis of this defective criterion, i.e. 'number per sq. in.' Trehan has compared the different varieties of cotton with respect to whitefly infestation but the flaw will be evident if one just considers how much the different varieties of cotton differ in their leaf area. Thus on page 57 (op cit.) the following conclusion is reported: Taking the entire season the whitefly attack on desi cotton was significantly higher than on the American cottons during both the years.' Now probably the total leaf area of desi cotton per acre may be less than that of American cotton and therefore even if the population of whitefly per acre be the same in the two varieties the number per sq. in. may come out more in desi cotton than in American cottons, but this difference in number per-sq. in. will not show any varietal preference shown by whitefly or varietal susceptibility of cotton. No such difficulty would have arisen if the different varieties of cotton were compared by comparing the population of whitefly per acre in the different varieties. This author has also reported in the same paper his conclusions regarding the effects on whitefly infestation of various other factors, e.g. irrigation, pH value, cultivation conditions, etc. and all these conclusions are based on 'number per sq. in.' It is needless to explain further that the effects of these factors on the leaf area of cotton are bound to have either exaggerated or

masked the effect on whitefly infestation. 5. The list of papers containing the flaw can be increased to any length. We have very briefly analysed above just a few cases merely as illustrations. In the reports from various provinces the cane borer attack is given as 'percentage incidence with respect to number of canes and tillers which vary from season to season and variety to variety. We may add that similar mistakes are not uncommon in papers published in other countries. Wolcott and Martorell [1943] have expressed the incidence of Trichogramma throughout as percentage of its host number. Smyth [1938] has expressed the incidence of sugarcane borer as well as that of its parasite in 'percentage values'. Box [ 1938 ] has compared plant crop and ration crop of sugarcane for borer infestation and this comparison is also based on 'percentage incidence' only. The incidence has been reported to be lower in ratioon than in plant crop. Now it is not clear whether the number of borers was actually less in ration or the 'percentage

value' has come out to be less in ration due to there being larger number of canes in ration crop than in plant crop.

#### Conclusions

From the foregoing it should be clear that if we want reliable data on incidence of pest or parasite it should always be calculated with reference to some fixed scale. So far as the agricultural crop pests and the parasites of those crop pests are concerned, the unit area of the land on which that crop is grown provides a fairly good and constant scale. Thus taking the case of bollworm incidence, if instead of estimating 'percentage incidence' we estimate population per acre and suppose we get 10,000 bollworms per acre in the first observation, then this number cannot change in subsequent observations by any alteration in the condition of the crop unless the number of bollworms actually increases or decreases. It is hardly necessary to point out that population per acre can be easily computed by simultaneously estimating by ordinary sampling procedure the number of insects per plant as well as number of plants per acre and then applying simple rule of three. If the nature of the insect under study allows, population per acre can also be calculated by directly counting insects on small units of land area. In cases wherein this change advocated above is found impracticable on other considerations and the incidence has to be recorded as percentage of the material liable to be damaged then care should be taken also to record along with it the average population per acre of the host material so that the incidence of the pest or parasite may be ultimately referred back to the fixed scale of unit land area whenever comparison with future record is desired.

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### SOME FIELD OBSERVATIONS ON THE PRESENT CYCLE OF DESERT LOCUST (SCHISTOCER CA GREGARIA FORSK.) IN SIND

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THE present cycle of the desert locust, which started in 1940, is remarkable in more than one respect. For the first time this country was forewarned of the locust invasion [ Pruthi, 1940 ], and it was also definitely established that the heavy infestation of the summer, 1941, originated mainly from the swarms of foreign origin [ Pruthi, 1941 ]. A significant feature of this cycle was that it afforded the entomologists of the country an opportunity of directing and supervising the offensive against this pest in its home—the permanent breeding grounds; during previous cycles the battle against the pest had to be waged after it had invaded other areas, because breeding grounds had not been located in this country. From August-October, 1941, the Thar Desert, which constitutes the south-eastern portion of Sind, was the centre of one of the severest infestations ever recorded, and one great and incessant battle took place in this tract over a wide front of several hundred miles.

The battle against the pest seemed already a lost one in some parts of this desert, towards the middle of August, 1941, when the writer was sent by the Government of Sind to give technical assistance. Two years later, in 1943, he was again sent to the same area with a similar purpose. During the course of very extensive touring of several weeks in 1941 and again in 1943, it was possible to study under natural conditions the behaviour of the pest when it was at its worst.

#### THAR DESERT

The Thar Desert, a part of the so-called Great Desert of India, occupies an area of about ten thousand square miles. It comprises the entire Chachro, Diplo and Mithi talukas, great part of Nagar Parkar and Umerkot talukas, and part of Khipro and Sanghar talukas. It is a tract of rolling sand hills which rise up to 150 ft. and run mainly from north-east to south-west.

The annual rainfall varies from 7 to 14 in., and almost all of it is received between June to October. Cultivation depends entirely on rain. Bajri (Pennisetum typhoideum) is very extensively grown, sometimes mixed with til (Sesamum indicum) and moth (Phaseolus acontifolius). Besides these, guar (Cyamopsis psoralioides) is almost the only other crop. The people, however, depend more on the produce of their herds of cattle and flocks of sheep and goats than they do on their cultivated fields. The locust swarms arrive in this tract either just before or after the first few showers of rain.

#### IMMIGRATION OF SWARMS

During July and August, 1941, many swarms of vellow locust passed over parts of Sind; they generally came from north-west and went towards south-east. On two occasions swarms were observed flying at right angles to the direction of the wind while its velocity exceeded seven miles an hour. The first observation was made at Sakrand, on 28 The swarm was flying very low and July 1941. had segregated into thousands of units. Each unit consisted of 20-100 locusts, and all of them were following the same direction a short distance from one another. The locusts flew only short distances, may be 30-150 vd. and were then forced down to the ground by strong wind. After a few minutes they again took to wings and were forced down again a short distance ahead. This went on for over half an hour till the entire swarm passed away. A similar case was observed on 2 August 1941, at a village, Kak, 20 miles away from Khipro in Thar Desert. This type of flight was also observed by my colleagues twice.

The regular migrations of this locust in the solitary phase, eastwards from Baluchistan to Rajputana in May and June, and back from Rajputana to Baluchistan in October and November, has been fully established [Rao, 1940]. During May and June the general direction of wind, which is usually over 5-6 miles per hour, is from south and south-west and the pest flies partly against the wind because the direction of flight is from north-west, i.e. from Baluchistan to Rajputana side. Probably the pest mainly flies while the velocity of wind is low.

#### OVIPOSITION

In Sind, extensive egg-laying took place in the Thar Desert after the commencement of monsoon in 1941 and 1943, and to a lesser extent in other parts of Sind during spring, 1943. The Thar Desert received over 7 in. of rain during July and August, 1941 and the entire desert tract was very suitable for egg-laying. Swarms of locust invaded this desert from about the middle of July to the end of August and were observed pairing and egg-laying under diverse conditions—in the fields with standing crop, in fallow land with or without vegetation, near the base of perennial bushes and rarely in thick annual vegetation. The mounds of sand near the base of perennial bushes were preferred.

During February and March, 1943, egg-laying was reported from some non-irrigated tracts of Larkana and Dadu districts. This area is ordinarily most unsuitable for egg-laying because the soil is very hard, and there is little or no rainfall during winter or spring. The heavy floods during July, 1942, in North Sind had, however, flooded this barren tract and several swarms laid eggs in an area stretching over 60 miles.

During July, 1943, there were good showers of rain in parts of the Thar Desert, and egg-laying took place over a wide area. It was interesting to observe that a swarm had moved from north to south in Chachro and Umerkot talukas, over a distance of about 80 miles, and laid eggs all over the area. The egg-laying was heavy in the north but it steadily decreased southward and, in areas further south it, did not take place. The hatching of eggs, as was expected, took place first, on 10 August, in the north and on or about 20 August in the extreme south.

#### EMERGENCE OF VERMIFORM LARVAE

Vermiform larvæ were frequently observed in the process of casting their overalls. They were found lying around the emergence holes at a distance of about three inches. After casting the overalls the hoppers moved singly in all directions, and took shelter on the nearest bushes or annual plants.

#### FORMATION OF BANDS

The primary factor in the formation of bands appears to be, as pointed out by Uvarov [1928], deposition of egg pods in more or less dense groups and congregation of hoppers from the beginning of their life. Besides this factor, Uvarov is of the opinion that the response to light and warmth also play an important part. Over a large stretch of thousands of square miles in the Thar Desert, conditions of light and warmth are very similar and it is doubtful if these factors have any direct influence on the formation of bands.

One other factor which assists in the fusion of bands is the physical feature of the breeding areas. Two or more bands near each other, it was noticed, got accidentally fused while climbing a sand dune. Before climbing a dune the bands locate gradual slopes, and either in the process of locating or in following the same path, the bands fused. Bands coming down from two or more sand dunes to a common valley fused likewise. Special mention may be made of the formation of bands and their fusion noticed in a village in Chachro taluka between 14 and 16 August, 1943. On 14 August several thin streaks of 1st stage hoppers, only 2-3 days old, were noticed on 4-5 sand dunes. The following morning streaks of hoppers from these and some other dunes were observed coming down to a wide valley continuously for about seven hours. These streaks fused, as the day advanced, and assumed the form of a regular band. Again, on 16th several loose bands were observed coming from the same

direction and they congregated more and more in the valley.

#### COMPOSITION OF BAND AND ITS SIZE

Bands composed of hoppers of one or two instars were generally observed, but on several occasions bands of all the five instars, mostly of 3rd-5th instars, were also noticed. In almost all the big bands, some hoppers, hardly one per cent, of the solitary phase were found; and in bands of 5th stage a number of adults of gregarious or solitary phase were always present, marching with them and behaving like hoppers.

The size of bands varied considerably; while some bands occupied an area of a few hundred square feet, others extended over several square miles. Generally bands of 1st and 2nd stage hoppers were small and those of 3rd to 5th stage hoppers were large. Special mention may be made of two exceptionally big bands, one of 1st stage hoppers and the other mainly of 3rd to 5th stage hoppers. The former was noticed on 15 August 1943, and was about half a mile long and a quarter of a mile in width. Ballard, et al. [1932] have stated that bands of 1st stage hoppers make local migrations within hatching grounds, but this band and several others were observed migrating in body from their breeding areas. The other band was seen near Islamkot between 10 and 14 September, 1941. The width of the band varied from two to four miles and it continuously passed near and partly through the town for five days, in a chain broken only at the night time. Its length could not have been less than 10 miles.

Associated with the size of a band is the question of its density. In the morning and evening bands were generally very dense, most of the hoppers being hardly half an inch apart, but during the day the hoppers spread out and became an inch or more apart. At times it was not possible to say as to where one band ended and another started, because either hoppers of the fusing bands had not come quite close to each other or bands in heavily infested areas had segregated, after exhausting the vegetation, into many units that moved in all directions in the face of starvation. Some bands were also secondarily split up into several bands owing to the passing of cattle or a large number of hoppers being left behind for moulting. It is thus evident that a band is never composed of the same hoppers but is picking up and leaving behind a large number of hoppers during course of its journey.

Further, it is suggested tentatively that the term band may be used for those hoppers that follow the same direction, occupy an area of 200-400 sq. ft. (depending upon the stage) while the distance between most of the hoppers be not more than two inches

#### MOVEMENT OF BANDS

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Bands of hoppers were observed moving almost throughout the day, from about an hour after sunrise to an hour or so before sun-set and on a few occasions, even before sun-rise or after sun-set. Once only, on 5 September, 1941, three bands of 5th stage hoppers were noticed moving quite fast, 2-4 hours after sun-set, on a full moonlit night.

Bands were generally observed to follow the direction of wind: two exceptionally big bands referred to above also followed the direction of winds. Ballard et al. [1932] found similar correlation between the direction of wind and the movement of bands. Once on their march, the bands moved up and down the sand dunes that fell on the way, following always the path of least resistance.

The speed of hoppers varied greatly during the day. In the morning and evening, their movement amounted to slow crawling, while during the greater part of the day it varied between crawling and jumping. Ballard et al. observed that some hopper bands covered a distance of about five miles in a day. It was possible to make careful observations once when it was estimated that a band of 1st stage hoppers covered a distance of about three-fourth of a mile in a day.

#### Breaking of bands and formation of swarms

The breaking up of 5th stage hopper bands takes place gradually as those about to moult leave the band and prepare for ecdysis. The bands thus thin down. As this process goes on the proportion of adults increases. In big bands, the proportion of adults was observed to be only 1-5 per cent, in medium size bands 5-20 per cent and in small bands 20-50 per cent. Two very small bands seen on 5 September in a highly infested area consisted of almost cent per cent adults. La Baume [1918] also observed the latter type of bands in Moroccon locust and called them 'adult bands'. It is thus evident that the occurrence of adults bands is brought about by the gradual segregation and not as a result of large number of adults emerging simultaneously. Freshly emerged adults show gregarious instinct and join any passing band.

It could not be definitely ascertained as to how long the adults remained associated with the hoppers, probably not more than 2 or 3 days. Thereafter they segregated, as a rule individually. Their segregation from 'adult bands' was not observed. Adults, which were able to fly, left the band in various ways; either by remaining on bushes and plants in the morning, when hoppers resumed their march, or by climbing on bushes and plants that fell on their way, or by joining any swarm that passed over-head.

The segregation of adults on bushes appears to be the first step in the formation of a primary swarm, a condition resembling somewhat the emergence of hoppers after 'intermediate moult' and their going on to the bushes. The number of adults found on bushes varied with the population of full grown hoppers. The formation and hovering of small primary swarms near the place of their origin was witnessed 4-7 days after their emergence in September, 1941. As these swarms flew others joined them. After about a week of their emergence, loose swarms were observed, and their flight appeared to be a sustained one—the distance covered at a time being a mile or more.

#### THE BEHAVIOUR OF SWARMS

The swarms commenced migrating from the Thar Desert a little earlier than usual in 1941 because of fairly warm weather in September and October. An approximate idea of the number of days which these swarms took to reach to other parts of the country was formed by following the reports of The summer brood adults swarm movement. originated on a very large scale between the first week of September and the middle of October, 1941, and some of these passed over parts of Nawabshah district between 17 and 19 September. Thus they took about a fortnight, after their emergence to cover a distance of 150-200 miles and reach adjoining tracts of Sind. In the last week of September and throughout October, large number of swarms were reported from various parts of Sind. These probably originated during September and first fortnight of October and also took about a fortnight to leave their place of origin,

The influence of temperature on the flight of swarms has not been studied. Swarms of desert locust were, however, observed to fly at different temperatures. The actual temperature of the air at the level at which they were flying or the temperature of the body has not been determined, but the two extremes of temperatures in shade when swarms were noticed at Sakrand were 110°F. on 28 July, 1941 and 55°F. on 11 January, 1942.

In the evening, just before sun-set, swarms settle down on trees or bushes and resume their flight 2-3 hours after sun-rise the next morning. A swarm which settled at about sun-set in a forest near Sakrand on 17 October, 1941, was watched on the following morning from about 7 A.M., the time of sun-rise. The day was a bright one, but up to 8-30 hours the locusts remained on the top branches of kandi (Prosopis spicigera) trees and showed no sign of activity. Just after 8.30 hours, they became active and started flying downwards, either to lower branches of the trees or to other low growing trees, and then flew down to the ground. There was a continuous stream of locust to the ground up to 10 hours. Those that came to the ground first started flying soon after 9 hours, in the form of a thin swarm,

and an almost continuous swarm kept departing till 10.30 hours.

The temperature recorded in a thermograph, hardly a mile away, varied from 72°F. at 7 hours to 88°F. at 10 hours, while the relative humidity fell down from 76 per cent to 42 per cent during the same period.

#### FOOD OF HOPPERS AND ADULTS

Bhatia [ 1940 ] studied the food preference of the solitary phase of this pest in Thar-Rajputana tract and found that all the common plants were eaten: most of these plants were also eaten by hoppers and at times by adults of the gregarious phase. Even leave of ak (Calotropis gigantea), which Bhatia found were not accepted after 24 hours of stravation, were eaten by hoppers on 24 September 1941 when the day was very hot and the infestation very heavy. There seemed no difference between the food taken by hoppers and adults, but the latter showed special preference for ripe or nearly ripe grains of bajri.

Further, some very juicy fruits, such as water melon (Citrullus vulgaris) and musk melon (Cucurbita moschata), were found completely consumed; while on another occasion very dry products, such as bark and some outer portion of plants of rape (Brassica napus), its seed pods and grains, which contain very little moisture, were seen eaten. This is not very surprizing because even wool, as shown by Afzal Husain and Mathur [1936], is eaten to

obtain moisture.

Cannibalism was observed to be very common during the third week of April, 1943, in Dadu district. Several hundred hoppers, mostly in the act of moulting, and freshly emerged adults were found mercilessly eaten, usually from posterior end, by other hoppers. This phenomenon was neither observed nor was ever reported from the Thar Desert. The primary cause for cannibalism, as shown by Uvarov [1928], appears to be thirst. In Dadu district the vegetation was dry and the days very hot when this phenomenon was noticed, while in the Thar the vegetation was green and the days mostly cloudy during the period of infestation.

#### NATURE AND EXTENT OF DAMAGE

The damage caused by the locust in the Thar Desert was two-fold. They damaged the crop and the pasture lands. In 1941 the infestation was extremely heavy and crops and pastures suffered very much. In 1943, on the other hand, infestation was comparatively mild, and the damage was mainly done by hoppers.

During 1941 at least 50 per cent young crops were destroyed by hoppers in Diplo and Mithitalukas. The damage done in other parts of Thar, though not so marked at first, became very severe when swarms of pink locusts ate away almost all

mature grains. Taking this desert as a whole, the damage to crops in 1941 can safely be put down to three-fourth of the produce. Besides, all the herds of cattle which came for grazing from neighbouring areas had to be taken either to less affected tracts of the desert or some other area; this caused great hardship. No rough estimate for 1943 is possible because parts of the desert could not be visited.

#### Role of birds in locust control

Afzal Husain and Bhalla [1931] studied the bird enemies of this pest in the Punjab and emphasized the invaluable service they rendered by exterminating thin swarms and destroying hoppers that escaped onslaught by man. During the infestation of 1943, the role of some birds, especially Rosy Pastor, in these two directions was most clearly visible.

The following birds, recorded as enemics of locust by Afzal Husain and Bhalla, were observed in the Thar Desert during the period of locust infestation,

August-September, 1943:

(1) Indian Jungle Crow (Corvus macrorhynchos); (2) Common India House-Crow (Corvus splendens); (3) Indian Tree-pie (Dendrocita vagabunda); (4) Red-Vented Bulbul (Malpastes cafer); (5) Indian Robin (Saxicoloides fulicata cambaiensis); (6) Fantail Fly Catcher, probably White-Browned (Leucocirca aureola): (7) Great (Indian) Grey Shrike (Lanius excubitor lahtora); (8) Rufous-backed Shrike (Lanius schach erythronotus); (9) King Crow (Dicrurus macrocerus); (10) Rosy Pastor or Rose-coloured Starling (Pastor Roseus); (11) Common Myna (Acridotheres tristis); (12) Indian House-Sparrow (Passer domesticus Indicus); (13) Franklin's Crested Lark (Galerida cristata): (14) The Blue-Jay or Indian Roller (Coracias benghalensis); (15) Indian Hoopoe (Upapa epop orientalis); (16) Laggar-Falcon (Falco jugger); (17) Common Pariah Kite (Milvus migrans govinda); (18) Common Pea Fowl (Pavo cristatus): (19) Northern Grey Partridge (Francolinus pondicerianus).

Most of these birds were seen eating hoppers and some of them also adults. Of the above mentioned birds only six, viz. House Crow, Indian Grey Shrike, Rosy Pastor, Common Myna, Indian House-Sparrow and Common Pariah Kite were very common. None of the birds except Rosy Pastor attacked the pest in flocks. This bird was the commonest and probably destroyed more locusts than all other natural

enemies put together.

Rosy Pastor is a regular visitor of Thar It generally comes during September-October when bajri crop matures and feeds on grains. During 1941 and 1943 flocks of this bird came at least one month earlier, soon after swarms of yellow locust started coming, and probably came in much larger numbers than in previous years. These flocks were noticed feeding exclusively on hoppers of all the

stages and adults, in almost all the infested areas. The flocks generally consisted of 20-30 birds, but on a few occasions as many as 100 birds were observed. While attacking bands, usually large number of flocks perched together on a few bushes or trees near them.

The role of this bird in reducing the very severe infestation of 1941 could not be fully evaluated, but in 1943 it was quite appreciable. From about the middle of August, 1943, flocks were observed feeding on hoppers, almost from the time of their emergence, in places wide apart; and the flocks increased in number and size as the time advanced. In spite of the good organization set up well in time, during 1943, adults started emerging, in some places up to 10 per cent of the hoppers population, from about the first week of September in Chachro taluka. Almost all of these and in addition a large number of 5th stage hopper bands were completely destroyed by this bird, and no swarm emerged from this desert tract. A number of supervisors of heavily infested areas frankly admitted that they could not, but for the invaluable help of this bird, effectively destroy, with the labour at their disposal, the pest in time. While in mildly infested areas control parties were disbanded a week or more earlier than expected because of the havoc wrought by this bird.

It was difficult to form any definite idea about the population of Rosy Pastor, but on one occasion, while passing through a heavily infested area of about 3 miles  $\times \frac{1}{4}$ th of a mile, on 12 September, 1943, the writer saw not less than fifty thousand

The Common Pariah Kite was seen only once in very large numbers—not less than five thousands in one square mile. They were eating hoppers and freshly emerged locusts on the ground and bushes. They, however, attacked the bands individually and not in the form of a flock.

Some other birds, (1) White Cheeked Bulbul (Malpastes leucogenys), (2) Ashy Crowned Finch-Lark (Eremopteryx grisae), (3) White-backed Vulture (Psuedogyps begalensis), (4) Tawny Eagle (Aquila rapax) and several types of Doves, were also found, but they were not observed feeding on locusts and are neither recorded as their enemies.

#### SUMMARY

The observations made on the behaviour of the desert locust during the present cycle, mainly in the Thar Desert, are described.

In their east-ward migration in summer months. swarms may fly at right angles to wind when the wind velocity exceeds even seven miles per hour.

The physical features of the Thar Desert, besides other factors, play an important part in the initial formation of a band or in the fusion of two or more bands. Bands composed of hoppers of one or two stages are generally found but at times they are composed of three or even all the five stages.

The size of bands varies considerably; bands composed of young hoppers are smaller than those of older hoppers. Small clusters of hoppers, occupying less than 200-400 sq. ft. should not be called bands. Freshly emerged adults are found in bands of 5th stage hoppers and move with them for some time. Bands follow the main direction of wind; in going up or coming down a sand dune, they follow the path of least resistance. Bands composed of hoppers of 5th stage break gradually. The proportion of freshly emerged adults increases with the decrease in size of these bands, and often adult bands' are formed.

Adults segregate from bands individually and rest on bushes. About a fortnight after their emergence, they may leave their place of birth and cover a distance of two hundred miles or more.

Pink locusts and hoppers eat almost all desert plants, the former show special preference for grains of bajri. Cannibalism was observed only under hot and dry conditions.

A large number of birds eat hoppers and adults. Of these, Rosy Pastor alone attacks the pest in flocks, and is a very important agent in reducing the severity of infestation.

#### ACKNOWLEDGEMENTS

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## STUDIES ON EARIAS SPECIES (THE SPOTTED BOLLWORMS OF COTTON) IN THE PUNJAB

IV. THE HOSTS AND HOST-PREFERANCE OF EARIAS CUPREOVIRIDIS WLK., E. FABIA STOLL. AND E. INSULANA BOISD.

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EARIAS FABIA and E. insulana have been known as serious pests of cotton in India for forty years as serious pests of cotton in India for lotely years [Lefroy, 1906, 1909]. E. cupreoviridis, is a pest of cotton in China [Li, 1937], but is not known to attack cotton in this country, although it is found on other Malvecous plants. Fletcher [1917, 1919], Fletcher and Misra [1919], Afzal Husain [1925, 1929], Fletcher and Misra [1919], Afzal Husain [1925, 1929] Chopra [1928], and Deshpande and Nadkarny [1936] have recorded the host plants of these species in various parts of India, but there is no precise information with regard to the plants on which the different species subsist. Sometimes vague statements, based on insufficient data, have been made. Richards [1924], for instance, states that all the three species are found on Abutilon indicum and cotton. No other worker has so far recorded E. cupreoviridis from either of these plants in India. Precise information concerning the food plants of these insects is of very great importance, because the destruction of alternative hosts during the close season, has been recommended as the only effective method of the control of Earias in Egypt [Dudgeon, 1916; Gough, 1919], South Gujrat [Deshpande and Nadkarny, 1936] and, by the senior author, for the Punjab.

To ascertain fully the part played by the different host plants in the carry-over of these insects, and the plants preferred by each species, detailed observations were made for three years, 1934-36, at Rohtak, Ludhiana, Sialkot, Lyallpur and Multan, representing the various cotton growing tracts of the Punjab. Caterpillars were obtained from flower-buds and pods of various host plants at 7-14 days interval throughout the season and bred in the laboratory.

#### DISCUSSION OF DATA

Of the 18 or so host plants recorded from various parts of India, the following 11 plants were observed to harbour *Earias* spp. in the Punjab:

- 1 Abutilon indicum.
- 2. Althea rosea.
- 3. Hibiscus cannabinus.
- 4. Hibiscus esculentus.
- 5. Malva parviflora.
- 6. Malva sylvestris.
- 7. Malvestrum tricuspidatum.

- 8. Sida cordifolia.
- 9. Sida humilis.
- 10. Sida rhombifolia.
- 11. Urena lobata.

The distribution of the species was studied on the first eight of these plants. The material obtained from the last three plants was not sufficient for

drawing any conclusion. Abutilon indicum. It is a perennial weed, usually found in waste land and is very common in southeastern, central and sub-mountainous regions of the Punjab. Except during the severe spell of summer, May-June, it remains green practically throughout the year, but bears flower-buds and pods mainly during February-April and September-November. In moist or shady places it flowers and fruits throughout the greater part of the year, except from about the middle of May to the end of August. Table I gives the number of moths bred from this plant during different months. The moths bred were invariably E. insulana. Even during the months of September or October when E. cupreoviridis and E. fabia were very common on other host plants, not a single moth of these two species emerged from the large collections made from this plant.

Abutilon spp. have been observed as the most favourite hosts of E. insulana by King [1918], and Bedford [1931] in Sudan, and their use as trapcrop has been recommended. It may be that Abutilon spp. are the original food plants of E. insulana, and this view is supported by the fact that one of us collected from a species of this plant a large number of caterpillars of E. insulana, during summer 1942, in the Thar Desert, some 70-100 miles from the nearest cotton fields. Further, in countries, such as Iraq and Sudan, where cotton was introduced E. insulana was one of the pests observed soon after its introduction, which shows that this insect was present on some wild plants, probably Abutilon.

Malva partiflora. It is a common annual weed found in very moist and shady places, such as gardens, banks of canals and grassy plots. It hears flower-buds and pods from about the end of January

to about the middle of May.

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Table I

Number of Earias insulana moths bred from Abutilon indicum

	Roht	nk		Ludhia	na		Sialkot		Lyallpur	Mu	ltan
Year	1934 1935	1936	1934	1935	1936	1934	1935	1936	1934	1935	1936
January		179						7	•••	• •	
March	108	260		122	38	••	8	6	71		
April	92 238	355	4	197	95	220	24	53	117	9	•••
Мау	42 10	265	87	38	••	134	79	72		46	45
September		74	372	38	56	158	16	84		13	3
October	.,	77	53	80	28	216	134	88		3	•
November December	} 3	152	116	28	12	168	161	66		17	

TABLE II

Number of Earias insulana moths bred from Malva parviflora

		Ludhian	a	Sial	Layllpur	
Year	1934	1935	1936	1934	1936	1935
March .		39	23	18	7	
April		25	12		136	11
Мау	34	15			11	

This plant is rarely attacked during the months of January or February, the part of the season when it is flowering. Of the 325 moths bred from M. parviflora, all were E. insulana. It is possible that this plant escapes the attack of the other two species of Earias because they are rarely found during the period when it flowers.

Malva sylvestris. It is a rare plant which was found growing wild in some moist places at Ludhiana. It bears flower-buds and pods mainly from April to July. Only E. insulana moths were bred from the collections made (Table III).

TABLE III

Number of Earias insulana moths bred from Malva
sylvestris

	Period		No. of moths
June 1934 May 1935			15 13
June 1935			16

Hibiscus cannabinus. It is grown for fibre, often around sugarcane or cotton fields in some parts of the Punjab. It is usually sown in May or June, and bears flower-buds and pods from August to November.

Of the 166 moths bred, all were E. insulana.

TABLE IV

Number of Earias insulana moths bred from Hibiseus cannabinus

Station	Period	1934	1935	1936
Sialkot . Ludhiana . ,,,	October September October November	3 38 12	32 19	15 47

From the four plants dealt with so far, only E. insulana has been bred. All these except Hibiscus cannabinus are wild plants.

Hibiscus esculentus (bhindi). It is a cultivated plant which is extensively grown as a vegetable crop, particularly in the vicinity of towns. It may be sown at any time from March to June and bears flower-buds and pods mainly from June to November. Table V gives the total number of moths bred and the proportion of the species emerging during different months.

It is interesting to find that all the three species subsist on this plant. E. fabia was very common from July to October and its proportion was generally over 70 per cent and at times even cent per cent; E. insulana, on the other hand, was very common

during May, June and November. E. cupreoviridis was bred only during July, August and September mainly at Lyallpur and Sialkot, and its proportion was generally less than 10 per cent. Only once it was bred at Ludhiana. It was not obtained from Rohtak and Multan.

It has been shown [Harcon Khan, 1944] that the population of *E. fabia* rises suddenly on cotton in some parts of the Punjab during July and falls down rapidly in November. It is interesting to observe similar fluctuations in the population of this species on *bhindi* also. Further, *bhindi* 

Table V

Number and proportion of Earias species on Hibiscus esculentus

		Roh	tak			Ludh	iana			Sialko	t			Ly	allpur			Mul	tan	
Period -	No. of moths	Percentage of insulana	Percentage of fabia	Percentage of cupreoviridis	No. of moths	Percentage of insulana	Percentage of fabia	Percentage of cupreoritidis	No. of moths	Percentage of	Percentage of fabia	Percentage of cupreoviridis	No. of moths	Percentage of insulana	Percentage of fabin	Percentage of cupreoritidis	No. of moths	Percentage of insudana	Percentage of fabia	Percentage of
									19	34										
une	1				17	100	0	0.				1				••	••		•	
uly					6	83-3	16.7	0						1		•	••			
August		1			231	0	100	0									••		•,•,	
eptember					85	1.2	98.8	0							••			.,		٠.,
ctober					67	100	0	0	86	32.5	67.5	0					• •	••	••	٠,
ovember					18	94.4	5.6	0									••			
ecember	1		l	٠.	7	100	0	0	1	1	1		l		۱.,				۱	١.
									19	35										
ay .					125	100	0	0	8	100	0	0								
ine .			<b>-</b>		187	99-5	0.5	0	10	60	0	40								
ıly .					230	30	. 70	0					61	11.5	80.3	8.2	11	9.9	9.1	
ugust	34	8.8	91.2	0	281	4.9	90.8	4.3	87	13.8	67.8	18-4	310	4.5	94-2	1.3	7	28.6	71.4	
optember	38	10.5	89.5	0	228	8.3	91.7	0	80	3.8	88.7	7.5	350	1.1	98.9	0	78	15.4	84.6	
otober	21	9.5	90.5	0	107	92.5	7.5	0	20	60	40	0	191	41.4	58.6	0	9	11.1	88.9	
ovember					13	100	0	Ö	11	100	0	0	41	82.9	17.1	0	19	89.5	1.0-5	
ecember		١				١			10	100	0	0		١					۱ ا	
									193	6										
ay .		1			11	100	0	0 1		ı			24	91.7	8.3	0				
ay					32	100	0	0					95	59-1	39.8	1.1				
ily .					204	0.5	99.5	0	31	0	93.5	6.5	88	7.9	85.3	6.8	46	10.9	89-1	
ngust	43	16.3	83.7	o	102	0	100	0	90	0	86.7	13.3	289	0	99-3	0.7	50	2.0	98-0	
	25	12.0	88-0	0	36	0	100	0	62	0	96.8	3.2	237	1-3	98.7	0	52	9.6	90-4	
ptember ctober	35	11.4	88-6	0	98	27.6	72-4	o	26	23.1	76-9	0	154	16.9	83.1	0	28	42.9	57.1	
ovember					31	67.7	32.3	0	36	52.8	47.2	0	97	70-1	29.9	0				

appears to be the most favourite host plant of *E. fabia*, because it was found in much larger numbers on this plant than on cotton or any other plant all over the province. *E. insulana*, on the other hand, was not common on *bhindi* during the period cotton was bearing large number of flower-buds and bolls—July to October, and the high proportion of this

species on bhindi during certain months was partly due to the scarcity of food on cotton and partly to the low population of E. fabia in the field.

The cultivation of bhindi as a trap crop around

The cultivation of bhindi as a trap crop around cotton fields has been recommended as a measure of control by a number of workers, Lefroy [1906], Fletcher and Misra [1919] and Jhaveri [1921].

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Since only E. fabia is found on this host in appreciable numbers during the main cotton season, it is obvious that this measure will be effective only in places where E. fabia is the chief pest.

Althea rosea (Hollyhock). It is a cultivated and a common ornamental plant grown in gardens and parks, usually near the towns. It is sown at any time from October to January and bears flower-buds and pods mainly from March to June.

Table VI

Number of Earias species bred from Althea rosea

	-					<del></del>				1				<del></del>			
							Ro	htak			Ludl	nian <b>a</b>			Siall	kot	
	1	Perio	d			No. of moths	No. of insulana	No. of fabia	No. of cupre- oviridis	No. of moths	No. of insulana	No. of fabia	No. of cupre- oviridis	No. of moths	No. of insulana	No. of fabia	No. of cupre-
				14 th					1934								
April .	•		•	•	•		••			••	••	••		10	10	0	0
May .	•	•		•			••	••	••	31	31	0	0	5	5	0	0
June .	• • •		•		•		••	• •		23	21	0	2	1	1	0	0
August		•			.		••	••	••	3	0	3	0		••		
									1935								
March .						127	127	0	0	28	28	0	0				
April .	•					63	62	1	0	15	15	0	0	••		••	
May .		•		• • • • • •		103	100	3	0	192	183	0	9	• •		• •	••
June .		•		•	•	28	28	0	0	29	29	0	0		•••	••	•
									1936 .								
April .					• 1	48	44	4	0	18	18	0	0	• •		••	
May .	•		•	•		52	47	5	0	19	18	0	1				

From the above it is evident that this plant harbours all the three species. Of the 785 moths bred, 12 were *E. cupreoviridis*, 16 *E. fabia* and the rest *E. insulana*. Fletcher [1917] states that this is not a favourite food plant of *E. fabia* and *E. insulana*. His finding seems to be substantiated by the fact that a number of small caterpillars were found dead in the gummy substance coming out of the wounds caused by their feeding.

It is interesting to find that the two cultivated plants, *Hibiscus esculentus* and *Althea rosea*, harbour all the three species. Does it indicate that through domestication they have lost characters which make certain other plants distasteful to some of the species of *Earias*?

Malvestrum tricuspidatum. It is a perennial weed, commonly found in moist and shady places. It usually bears flower-buds and pods from May to November, but near some well irrigated plots the plant may bear them all the year round.

Of the 907 moths bred, 591 or just over 65 per cent moths were *E. cupreoviridis* and the rest *E. insulana*. From July to October, majority of the moths that emerged were *E. cupreoviridis*, while during other months *E. insulana* exceeded in number

Sida cordifolia. It is a small perennial weed found in waste lands, and bears flower-buds and pods generally from July to November. It is found mainly in the South-eastern Punjab.

Table VII\*

Number of Earias species bred from Malvestrum tricuspidatum

		Rohtak			Sialkot		Lyallpur			
Period	No. of moths	No. of insulana	No. of cupre- oviridis	No. of moths	No. of insulana	No. of cupre-oviridis	No. of moths	No. of insulana	No. of cupre-oviridis	
					1935	<u> </u>				
May June July August September October- November	··· ·· ·· ··	•••		4 3 11 5	0 0 1 5	4 3 10 0	43 10 87 49 24	39 10 7 0 0	4 0 80 49 24	
					1936					
January April May June July August September October November December	69 25 17 26 29 4 21	67 13 2 0 0 0 0 21 42	2 12 15 26 29 4 0	13 6  46 5 5 3	15 13 6  0 2 3 0 1	0 0 0  46 3 2 3 0	143 37 45 38 53 4	31 0 0 6 6 2	112 37 45 32 47 2	

\*9 moths, all E. insulana, were bred at Multan during October-November, 1935 15 moths, all E. insulana, were bred at Ludhiana in September-October, 1936

TABLE VIII

Number of Earias species on Sida cordifolia

Station	$P_{ ext{eriod}}$	No. of moths	No. of insulana	No. of cupreoviridia
	September 1934	20	2	18
Ludhiana	October 1934	13	1	12
	May 1935	2	0	2
	August-September 1935	3	1	2
되지 않는 사람들이 하는 살이 얼마나 되었다.	October 1935	40	3	37
	November 1935	5	0	5
	September 1936	67	0	67
	July 1936	85	0	85
Rohtak · · ·	August 1936	115	0	115
	September 1936	12	0	12 .
Sialkot .	October-November 1936	20	0	30

Mostly E. cupreoviridis moths were bred from this host; of 382 moths that emerged only 7 were E. insulana.

These two wild plants, M. tricuspidatum and S. cordifolia, are favourite hosts of E. cupreoviridis.

#### Summary

Althea rosea and Hibiscus esculentus, both cultivated plants, are common hosts of all the three species of Earias.



E. cupreoviridis is mainly found on Malvestrum tricuspidatum and Sida cordifolia, both wild plants, and only occasionally on the two common host plants; E. fabia is mainly found on Hibiscus esculentus and to a small extent on Althea rosea, while E. insulana is found on almost all the host plants, but very likely prefers Abutilon indicum most.

E. cupreoviridis is not common in the Punjab;

nor are its host plants common.

E. fabia is very common from July to October when its favourite food plant-bhindi-is bearing large number of pods. It is possible that it attacks cotton when its population has increased considerably on bhindi.

E. insulana is the commonest species in the province. It seems to be least specialized with regard to its host plants and its wide distribution in many countries in Africa and Western Asia may partly be on account of its adaptability to subsist on several hosts.

#### ACKNOWLEDGEMENTS

This investigation was a part of the Punjab Bollworm Scheme, financed by the Indian Central Cotton Committee, and we express our gratitude to the Committee. We record our grateful thanks to Mian Afzal Husain, under whose guidance this work was conducted, for his constant help, and for going through the manuscript and making valuable suggestions. We are also indebted to Messrs P.M. Verma and Ghulamullah for their help in the collection of data at Rohtak and Multan, and to the Imperial Entomologist for giving facilities in the final revision of this paper.

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#### RESEARCH NOTE

A PRELIMINARY NOTE ON THE DISTRIBUTION OF FRUIT PULP IN CITRUS SQUASHES

By GIRDHARI LAL, Ph.D. (LOND.), D.I.C., Fruit Biochemist, and NAGINA LAL JAIN, M.Sc. (TECH.), Research Assistant, Fruit Products Laboratory, Lyallpur

(Received for publication on 31 August 1944)

It is a matter of common experience that fruit pulp in bottled citrus squashes (foreign or local made) partly settles at the bottom and 'partly floats on top. When the pulp floats on the top it presents a very unsightly and clumpy appearance and reduces considerably the market value of the product. This difficulty has been sought to be remedied by manufacturers in this country, particularly in the case of lime squash, in which most of the pulp in the form of a thick oily emulsion has a tendency to float on the top of the bottled product. A detailed study of the problem was undertaken by the authors under the Special Fruit and Vegetable Preservation Scheme, Lyallpur, financed by the Imperial Council of Agricultural Research.

Preliminary experiments were carried out in case of lime squash. In all, 46 different sets of this squash were prepared (according to the usual formula, 1 lb. juice,  $1\frac{1}{4}$  lb. sugar and  $\frac{3}{4}$  lb. water) with the following treatments:

- 1. Different methods for extraction of juice, viz.
  (a) with hand juice extractors and, (b) in a basket press.
- 2. Thorough shaking of the squash and of the juice before preparing squash, to break the colloidal nature of the pulp.
- 3. Shaking of squash and of juice before preparing squash for 24 hours in a mechanical shaker.
- 4. Juice passed through fine holes under vacuum before preparing squash.
- 5. Heating the juice at 60°C. for 5-15 min. before preparing squash.
- 6. Heating the prepared squash at  $60^{\circ}$ C. for 1-15 min.

- 7. 'Flash' pasteurization of juice before preparing squash.
- 8. Placing the bottles of squash lengthwise and upside down.
- 9. Centrifuging the juice in a tube centrifuge, before preparing squash.
- 10. Addition of the following chemicals (with the range given in each case) to the juice before preparing squash:
- (a) 0.025 to 0.1 per cent common salt, (b) 0.025 per cent calcium chloride, (c) 0.05 to 0.5 per cent gum tragacanth, (d) 0.05 to 0.5 per cent solid pectin, (e) 0.5 to 1.0 per cent gum acacia, (f) 12.5 to 75.0 per cent by weight of a water extract prepared from the rag of limes left after juice extraction. (An equal amount of water was reduced in the formula in this case.)

Out of the above treatments, the heating of the prepared squash at 60°C., addition of substances like gum tragacanth, pectin, common salt, gum acacia and rag extract separately to the juice (before preparing squash), made the pulp settle at the bottom. By an addition of varying amounts of rag extract, it has also been possible to keep the entire pulp uniformly distributed throughout the product, a procedure which yields a cloudy squash in which no separation of pulp takes place. None of the above treatments imparts any undesirable taste or flavour to the product.

Experiments are also now under way, in which the results of the above investigation are being extended to other citrus squashes like those of grapefruit, lemon, Malta and Sangtra. Encouraging results have been obtained in this connection. The detailed results of the investigation will be published as soon as storage trials of these products have been completed.

### PLANT QUARANTINE NOTIFICATIONS

The following Quarantine regulations have been received in the Imperial Council of Agricultural Research. Those interested are advised to apply to the Secretary, Imperial Council of Agricultural Research, New Delhi.

#### NOTICE No. 2 of 1945

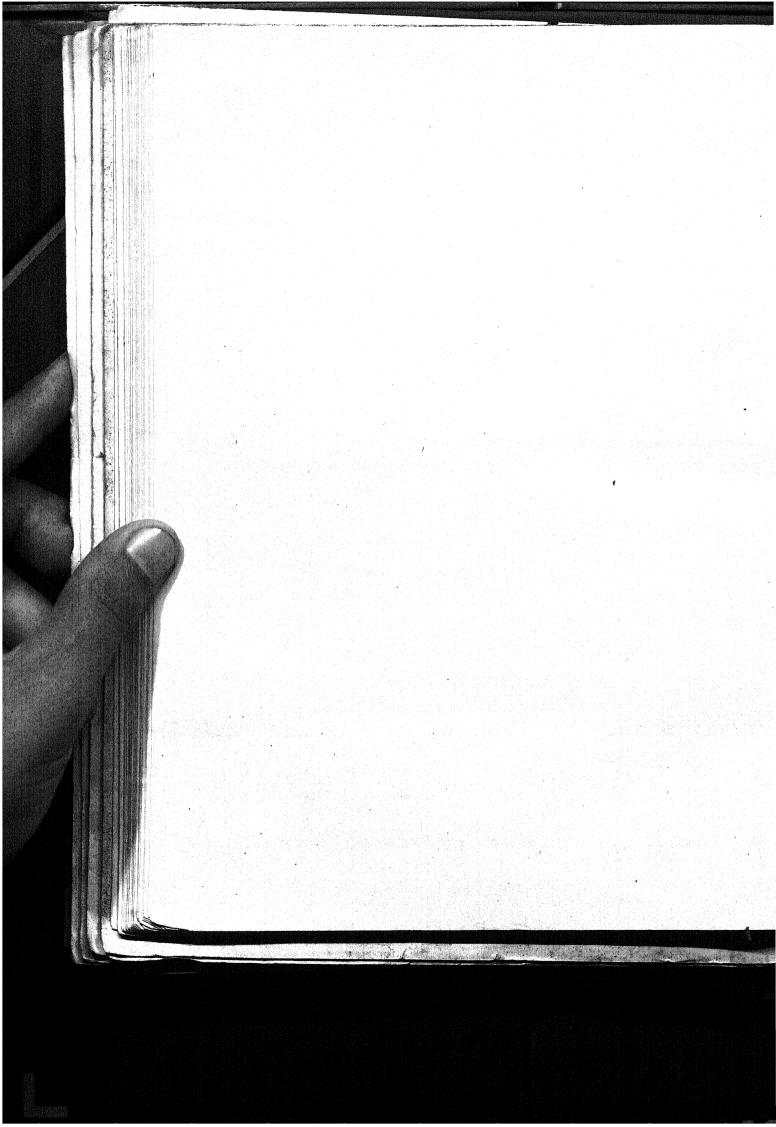
- Mexican Fruitfly Quarantine—B.E.P.Q. 64
  issued by the United States Department of
  Agriculture.
- Plant Quarantine Import Restrictions of the Presidency of Antigna, B.W.I. dated 20 January 1945, issued by the United States Department of Agriculture.

Notice No. 3 of 1945

- Service and Regulatory Announcements July-September 1944 issued by the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.
- 2. White-Fringed Beetle Quarantine (Quarantine No. 72) issued by the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.
- 3. Bureau of Entomology and Plant Quarantine—479, Supplement No. 1, Revised, issued by the United States Department of Agriculture.

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#### ORIGINAL ARTICLES

# STUDIES IN THE PERIODIC PARTIAL FAILURES OF THE PUNJAB-AMERICAN COTTONS IN THE PUNJAB\* XIV. MINERAL METABOLISM OF NORMAL AND

TIRAK-AFFECTED PLANTS

By R. H. DASTUR † and ABDUL AHAD, Punjab Agricultural College, Lyallpur (Received for publication on 3 April 1944)

(With 15 text-figures)

THE causes that give rise to the physiological disorder populary known as tirak in the Punjab 4F American cotton plants have already been described in the previous contributions [Dastur, 1941; Dastur and Samant, 1942]. The main symptoms of tirak-affected plants are premature shedding of leaves and immaturity of seeds in the bolls. The premature yellowing of leaves on light sandy soils and drooping of leaves on soils with saline subsoils were found to occur in addition to the above-mentioned symptoms. It has been established that nitrogen starvation was associated with tirak-affected crop on light sandy soils, while unavailability of water produced tirak symptoms in cotton crop that came on soils with saline subsoils. Thus two different physiological causes led to the development of a common symptom, viz. immaturity of seeds. It has been demonstrated that yellowing of leaves on light sandy soils was associated with low nitrogen contents and development of tirak symptoms could be prevented by application of nitrogen in the form of the sulphate of ammonia [Dastur, 1941]. It was also shown that when nitrogen was applied to such soils there was an increase in the nitrogen content of leaves along with the absence of tirak symptoms [Dastur, 1941].

Though the chemical basis of premature yellowing and shedding of leaves was known, it was not known how the immaturity of seed and lint was caused. The chain of events that led to the immaturity of seeds and lint had to be therefore determined. Premature yellowing of leaves in plants on soils with saline subsoils did not occur even though the seeds remained

immature on this type of soil as well. It was apparent that a physiological drought brought about the drooping and ultimate shedding of leaves on saline soils but the causes that produced immaturity of seeds and weak lint remained obscure. It was therefore necessary to investigate the internal causes that gave rise to these symptoms.

Tirak symptoms begin to appear only when the plants enter the fruiting phase. Before that stage the plants appear normal. In order to study the trends in the various metabolic activities of the tirak-affected plants it was necessary to make a chemical study of the plants from the seedling stage up to maturity, and this could not be done until the fields where tirak occurred were first located. Similarly the two soil conditions promoting tirak had to be differentiated before the investigation could be proceeded with. The investigation on the physiological chemistry of tirak was therefore dependent upon and was linked up with the investigation on other aspects of this tirak problem.

#### MATERIAL AND METHODS

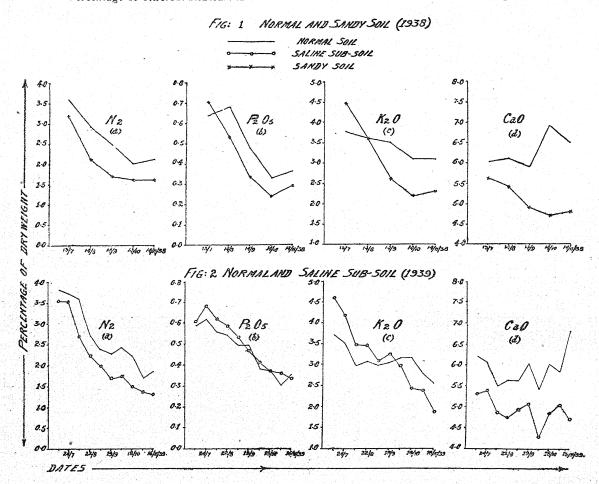
In order to study the mineral uptake in the normal and the tirak-affected cotton plants, the fields which were previously known to possess the following types of soils were selected at the Lyallpur Agricultural Farm in the cotton seasons of 1938 and 1939, viz. (1) normal sandy loam, (2) sandy loams with saline subsoils, and (3) light sandy soil with nitrogen deficiency. Five plant samples were taken from each soil type at random at monthly intervals in 1938, and at fortnightly intervals in 1939. Root, stem, all leaves and fruiting organs were separatly sampled and dried.

Similarly, samples of the leaves and bolls, etc. were also taken from a light sandy soil with nitrogen deficiency from the unmanured plots

<sup>\*</sup>The work reported in this paper was done in the Punjab Physiological (Cotton Failure) Scheme financed jointly by the Indian Central Cotton Committee and the Punjab Government

<sup>†</sup> Formerly Professor of Botany, Royal Institute of Science, Bombay

Percentage of different minerals in the leaves of 4F normal and tirak-affected cotton plants



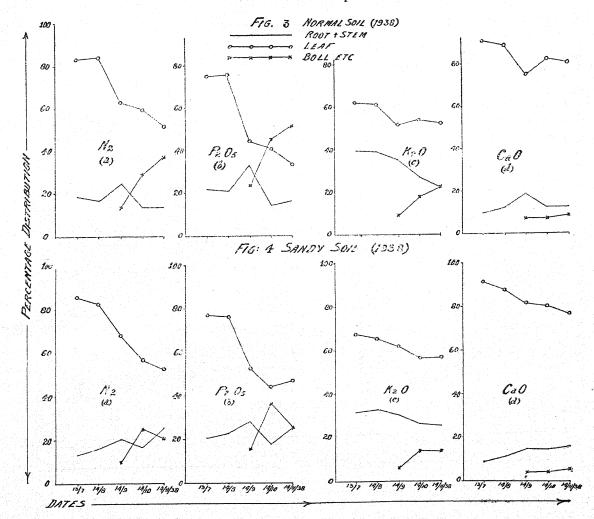
and from the plots where ammonium sulphate was applied. Samples were also taken at fortnightly intervals from the fields with normal soil and with soils with saline subsoils where cotton was sown at the usual sowing time in May and a month later, i.e. in June. The samples of developing bolls were taken at random from four plots of each of the three soil types at weekly intervals up to the eighth week of development in 1941. The first and the last samples were taken in duplicate from each soil type to see the variations due to sampling in the chemical composition of the material collected on the same day. The bolls were immediately weighed and divided into stalks, sepals, carpels, seed and lint. They were placed first in the electric drying oven, where a rapid draft of warm air was blowing at 45°-50°C and then they were placed at 70°C. till the weight was constant. A representative part of each sample was dried finally at 100° C. to constant weight for the calculations of dry weight. Remaining parts of the samples which were already dried at 70°C. were powdered finely in the power knife mill and kept in the stoppered bottles for chemical analysis.

Total nitrogen was estimated by the modified Kjeldahl method to include nitrate nitrogen Calcium was estimated by the standard oxalate volumetric method and potash by sodium potassium-cobaltinitrite volumetric method. Phosphoric acid was determined by phosphoammonium molybdate method.

#### INVESTIGATION

The nitrogen, potash, lime and phosphoric acid were estimated in the roots, stems, leaves and the fruiting organs every month in the 1938 cotton season from the normal and tirak-affected

Percentage distribution of different minerals in the different parts of 4F normal and tirak-affected cotton plants



plants. The concentration of each mineral in each organ was determined on percentage oven-dry matter

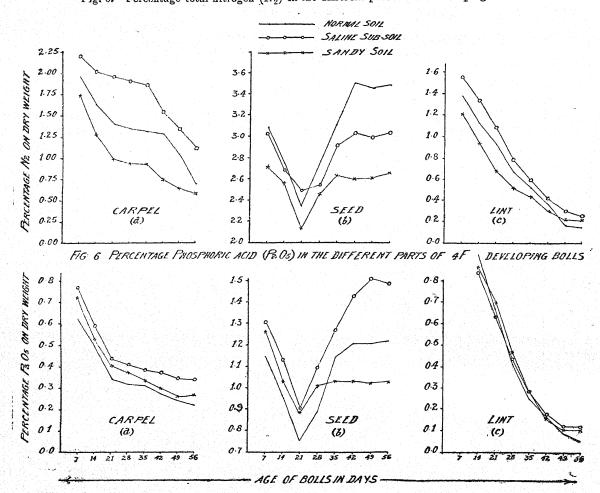
#### Mineral composition of leaves

The stems and roots of normal and tirak-affected plants did not exhibit any marked differences at any stage in their chemical composition. Marked differences were, however, noticeable in the leaves from normal and tirak-affected plants (Figs. 1 and 2). The deficiency of nitrogen and lime were found to occur in the leaves of tiak-affected plants from the early stages of growth, while the deficiency of potash became marked from the mid August, i.e. in the preblooming stage in the case of sandy soils and from about the end of September in the

case of saline subsoil. Phosphoric acid was low in the leaves of tirak-affected plants from light sandy soil only. The deficiency of nitrogen and potash was much more pronounced in the leaves of plants from light sandy soil than from the leaves of plants from the soil with a saline sub-soil.

When the percentage distribution of each element in the different parts, viz. root, stem, leaves and bolls was studied some important differences between normal and tirak-affected plants were found (Figs. 3 and 4). A comparison will show that the bolls of the normal plants contained more nitrogen (35 per cent of the total nitrogen absorbed by the plant) than the bolls of the tirak-affected plants, which contained only 20 per cent of the total nitrogen. Similar

Fig. 5. Percentage total nitrogen (N2) in the different parts of 4F developing bolls



differences in the phosphoric acid and potash distribution were found, while there was no difference in the percentage distribution of lime in the bolls. The decrease in the percentage distribution of nitrogen, phosphoric acid and potash in the leaves at the fruiting stage indicated that these substances travelled from the leaves to the fruiting parts, where they were found to increase.

#### Mineral composition of bolls

A study of the concentrations of nitrogen, potash, phosphoric acid and lime in the carpels, seeds and the lint of normal and tirak-affected bolls at different stages of development revealed the following trends:

The concentrations of nitrogen (Fig. 5a), phosphoric acid (Fig. 6a) and up to a certain stage only, of lime (Fig. 8a) decreased while the concentration of potash (Fig. 7a) increased

in the carpels as the bolls matured. The potash content of carpeis of tirak-affected bolls remain-

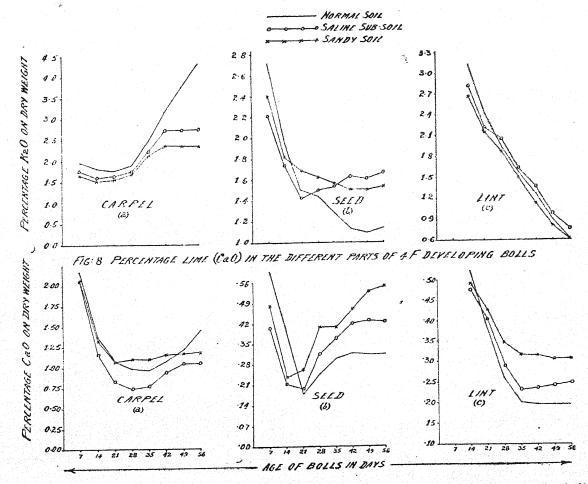
ed constant in the final stages of growth.

The concentrations of nitrogen (Fig. 5b), phosphoric acid (Fig. 6b) and lime (Fig. 8b) showed a fall in the seeds during the first three weeks of development after which an increase in their contents was found to occur except in the case of seeds from light sandy soils where the first two elements remained constant after the 5th week. Potash (Fig. 7b), on the other hand, showed a continuous decline in the seeds of normal bolls, while it remained almost constant in the seeds of tirak-affected bolls after the 5th week of development.

The concentrations of all the four minerals decreased in the lint as it matured (Figs. 5c, 6c, 7c and 8c)

The following were found to be important differences between the mineral contents of

### FIG 7 PERCENTAGE POTASH (K2.0) IN THE DIFFERENT PARTS OF 4F DEVELOPING BOLLS



normal and triak-affected plants on the two soil types:

1. A low nitrogen content at all stages of growth in the carpels, seeds and lint was a feature of tirak-affected bolls on light sandy soils (Figs. 5a, 5b and 5c). The carpels and lint of tirak-affected bolls on soils with a saline subsoil, on the other hand, showed higher nitrogen contents than the corresponding parts of normal bolls at all stages of growth (Figs. 5a and 5c). That was not the case with seeds (Fig. 5b) which like the seeds from light sandy soils contained less nitrogen than the seeds of normal bolls after the 3rd week of development.

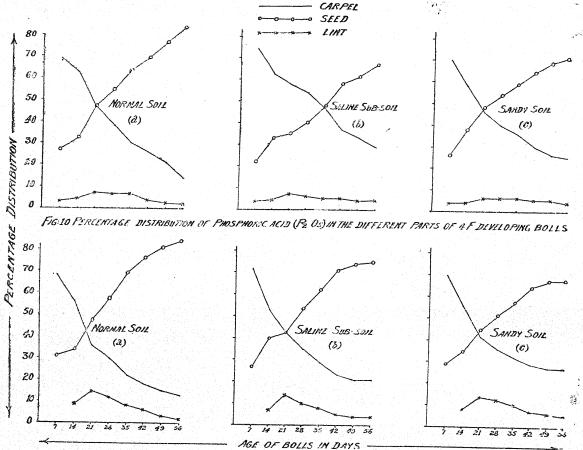
2. Phosphoric acid content was found to be below normal in seeds of tirak-affected bolls from light sandy soils in the last four weeks of boll development (Fig. 6b), while the same mineral was found to be present in larger con-

centrations than normal in the carpels of bolls from light sandy soils and in carpels and seeds from soil with saline subsoil (Fig. 6a).

The carpels of tirak-affected bolls were found to contain less potash at all stages than the carpels of normal bolls and this difference in the potash contents between normal and tirak-affected bolls became more pronounced in the final stages of growth (Fig. 7a). Potash content continued to increase in the carpels of normal bolls while it remained constant in the carpels of tirak-affected bolls during the last three weeks of boll development. The potash content of the seeds progressively declined in the normal bolls while it ceased to decrease in the seeds of tirak-affected bolls in the later stages of growth (Fig. 7b).

The lime contents of the seeds were higher in tirak-affected bolls than in the seeds of normal

FIG. 3 PERCENTAGE DISTRIBUTION OF TOTAL NITROGEN (N2) IN THE DIFFERENT PARTS OF AF DEVELOPING BOLLS



(bolls after the 3rd week of boll development (Fig. 8b). Similarly the lime content of lint was higher in *tirak*-affected bolls than in normal bolls Fig. 8c).

A low potash content in the carpels and a low nitrogen and a high potash and lime contents in the seeds were the common features in which tirak-affected bolls differred from normal bolls.

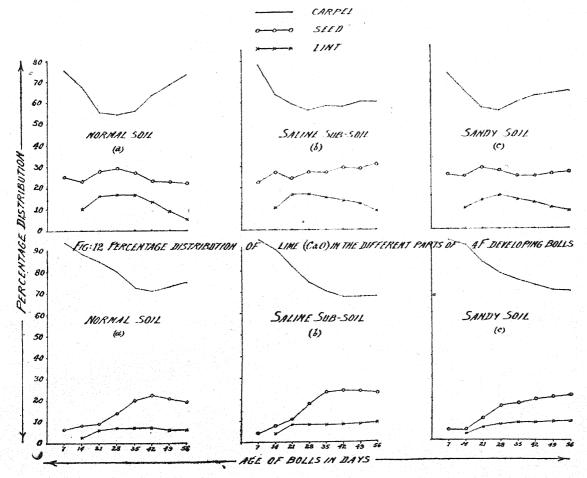
The graphs showing the percentage distribution of the four minerals in the different parts of the bolls of normal and tirak-affected plants are given in Figs. 9, 10, 11 and 12. The trends in the graphs are of two types, one type characteristic of nitrogen and phosphorus and the second type characteristic of potash and lime.

In the first type there was a progressive decrease in the percentage distribution of nitrogen and phophoric acid in the carpels, and an increase in the seeds as the bolls matured (Figs. 9 and 10). This held good for normal as well as

for tirak-affected plants. There was thus a migration of these substances from the carpels to the seeds. In the case of tirak-affected bolls a greater percentage of nitrogen and phosphoric acid remained in the carpels at maturity than was found to be the case with the carpels of normal bolls. The seeds of tirak-affected bolls thus contained comparatively lesser percentages of total nitrogen and phosphoric acid than the seeds of normal bolls at maturity.

In the second type of curves (Figs. 11 and 12) there was a decline in the percentage distribution of potash and lime in the carpels during the first four weeks of development in all the three cases, after which there was no further decrease. An increase in the percentage distribution of potash in the carpels of normal bolls was found to occur in the last three weeks of development. There was no increase in the potash content of the seeds as they matured and the curves for carpels and seeds did not consequently intersect

Fig. 11. Percentage distribution of potash (K20) in the different parts of 4F developing bolls



each other. An increase in the percentage distribution of lime in the seeds of all the three types of bolls was found to occur up to the 5th week of development but after that stage it remained constant. No difference in the percentage distribution of the four substances was found to exist in the developing lint of normal and tirak-affected bolls.

The stages of growth at which the maximum amounts of these four substances enter the bolls were determined by calculating the percentage of the total uptake of each substance every week (Fig. 13). The maximum percentages of total nitrogen, phosphoric acid, potash and lime were found to be present in the bolls at the end of the second week. The uptake of nitrogen in tirakaffected bolls declined after the second week while it continued to remain high in the normal bolls. The curve for the uptake of phosphorus showed two maxima in the case of normal and

tirak-affected bolls from saline subsoils, while the uptake of this mineral declined from the second week in the bolls from light sandy soils. The trends in the uptake of potash at different tages of growth of normal and tirak-affected bolls were similar to those discussed for nitrogen. The uptake of potash continued to be high up to the end in the normal bolls while it diminished after the second week of development in the tirak-affected bolls. No differences in the uptake of lime at different stages of development were found to exist between normal and tirak-affected bolls.

The uptake of nitrogen and the three minerals was found to be higher in first three weeks in tirak-affected plants than in the normal plants.

DISCUSSION

As immaturity of seeds occurred in two different soil types it was possible that the chain

FIG. 13 PERCENTAGE INCREASE OF THE TOTAL UPTAKE OF DIFFERENT NINERALS IN THE 4F DEVELOPING BOLLS.

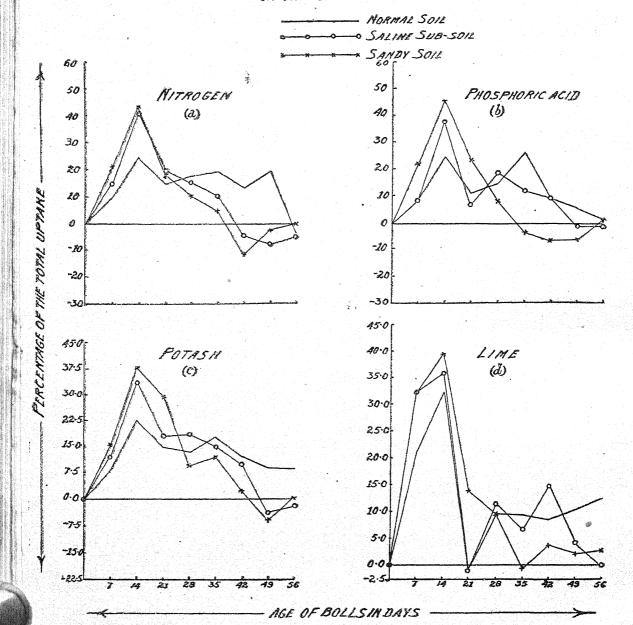


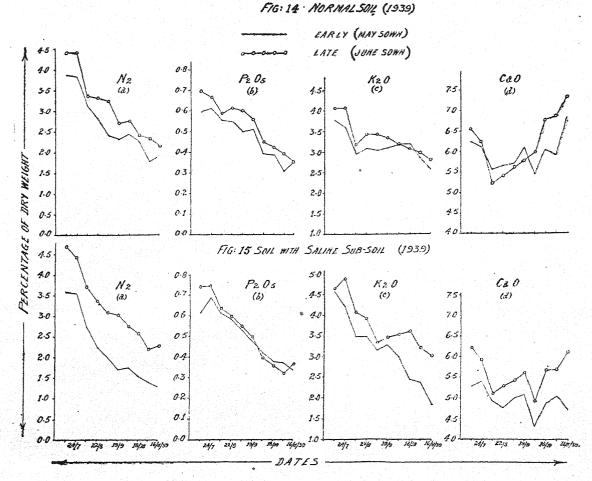
Table I

Percentage of potash in the leaves of control plants and of plants treated with 200 lb. of  $K_20$  per acre

	Saya ya	Re	plicate numbe	r			
Treatment	1	2	3	4	5	Mean	S. E.
Control 200 lb. of K20 per aere	2·82 2·96	3·25 3·27	2·92 2·63	3·49 3·64	2-97 2-85	3·09 3·07	0-059

III

Percentage of different minerals in the leaves of early and late 4F cotton plants on normal and saline subsoil



of events leading to the development of the common symptom on the two soils may be quite different. It may also be mentioned here that intensity of tirak, i.e. the degree of immaturity of seeds was greater on light sandy soils than on soils with saline subsoils. From the results discussed above it was clear that a greater disturbance in the mineral uptake was also noticeable in the bolls from light sandy soils

than in the bolls from saline subsoils.

It has already been demonstrated by Dastur [1941] that applications of nitrogen in the form of the sulphate of ammonia to light sandy soils ameliorated tirak occurring on such soils. It was also shown, though not very conclusively, that when nitrogen was applied, there was an increase in the uptake of potash and lime by the plants along with that of nitrogen It therefore appeared probable that the uptake of potash

and lime was influenced by the level of nitrogen in the soil. The deficiency of potash in the leaves and bolls and of lime in the leaves on light sandy soils may thus arise indirectly on account of deficiency of nitrates in the soil. It was therefore undertaken to establish this conclusion.

The leaves of the plants from plots in a field experiment where potash was applied at the rate of 200 lb. of K<sub>2</sub>0 per acre were analysed for potash along with the leaves of plants from control plots where potash was not applied. Five plant sample was taken for analysis from five control and five treated plots. The leaves from each plot were separately dried and analysed for potash.

Thus direct applications of potash did not increase its uptake, as no differences were noticed in the potash contents of the leaves of the control and treated plants In addition the measurements of boll weight (i.e. the weight of seed cotton per boll) indicated no increase in the maturity of seeds of plants in plots treated with potash. Thus tirak condition was not ameliorated.

The application of nitrogen in the form of the sulphate of ammonia was found to ameliorate tirak and to increase the yields. It was therefore undertaken to determine the potash contents of the leaves and carpels from the control plots and from the plots treated with 50 lb. nitrogen in the form of the sulphate of ammonia. The leaves of five plants each, from four unmanured and four manured plots were taken for analysis.

TABLE II

Percentage of potash in the leaves and carpels of unmanured plants and of plants manured with sulphate of ammonia in light sandy soils

	Les	ives	Carpels				
Plot	Unmanured	Manured with 50 lb. N	Unmanured	Manured with 50 lb. N			
].	2.36	3.26	3.20	4.27			
2.	2.76	3.22	3.04	4.62			
3.	3.06	3.76	3-11	5.28			
4.	2.63	3.64	3.26	4.86			
	2.70	3.47	3-15	4.76			
s. e.	( <u>+</u> 0·085)		( <u>+</u> 0·159)				

The potash contents of the leaves and the carpels of plan ts manured with 50 lb. of nitrogen in the form of the sulphate of ammonia were significantly higher than the potash contents of the leaves and carpels of unmanured plants. It has already been shown that when nitrogen was applied, the nitrogen content of the leaves of manured plants was significantly greater than the nitrogen content of the leaves of unmanured plants at the same stage of development [Dastur, 1941]. There was also an increased uptake of lime as revealed by the analysis of the leaves of manured and unmanured plants [Dastur, 1941]. The results clearly suggested that potash and lime were not deficient in the light sandy soils but their uptake was lessened on account of the deficiency of nitrates in the soil. If the deficiency of nitro-gen was made up by artificial application of nitrogen, the uptake of these minerals was

greatly increased. Knowels, Watkins and Cowie [1940] found similar increase in potash uptake in the potato plant when nitrogen was applied.

The applications of nitrogen to light sandy soils were found to increase significantly the bearing, i.e. the number of bolls per plant [Dastur and Mukhtar Singh, 1944]. Further investigations have shown that nitrogen had no direct relation with the seed maturity. In a field where the soil was light sandy as well as saline in the subsoil, i.e. where both tirak-promoting soil conditions were present, application of the sulphate of ammonia was found to increase the boll number per plant but did not reduce the immaturity of seeds as compared with boll number and seed immaturity found in the plants in the unmanured plots. The leaves of plants from treated plots did not exhibit the external symptoms of tirak, viz. premature yellowing and shedding but the bolls contained both partially and fully immature seeds [Dastur, Mukhtar Singh and Sucha Singh, 1944].

The application of the sulphate of ammonia to sandy loams (not deficient in nitrogen) with saline subsoils did not either increase the boll number or the maturity of seeds [Dastur and Mukhtar Singh, 1942] as compared with number and the seed maturity found in plants of the unmanured plots. Applications of potash did not also increase either the boll number or seed maturity. In no case application of lime was found to have any ameliorative effect on tirak on any soil type.

The results clearly indicated that there was no direct deficiency of potash or lime on the two soil types where *tirak* occurred. This fact was further confirmed by the determinations of exchangeable calcium and potassium from soils under normal crops and from soils under *tirak*-affected crops [Dastur and Samant, 1942]. No differences in either exchangeable calcium or potassium under normal and *tirak*-affected crops were found to exist.

A direct deficiency of nitrogen on light sandy soils reduced the uptake of potash on such soils. The case was different with soils with saline subsoils where a direct deficiency of nitrogen was not found to occur. In the former case immaturity of seeds was reduced by an application of nitrogen while that was not found to be the case in the latter type of soil. It therefore appeared probable that the immaturity of seeds was associated with the low potash content of the leaves and of carpels of tirak-affected plants.

The association of immaturity of seeds with low potash content has already been known to

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occur in case of other crop plants,—Russell [1937] has pointed out that if potassium was deficient, grains of cereals did not mature. Neal and Gilbert [1935] reported that the application of potash remedied the disease of cotton known as 'cotton rust' or 'potash hunger' where the seeds remained immature. Skinner and Pate [1925] found an increase in boll weight as a result of potash applications in fine sandy soils. A decrease in weight of the seeds was reported by Wood [1934] when potash was omitted from manurial experiments. Schuster [1927] reported that soyabean plants grown with deficient potash supply produced small and immature seeds with low oil contents.

The cotton plants on soils with saline subsoil showed symptoms of physiological drought at the fruiting stage. The leaves drooped and were gradually shed. Here it was not primarily a case of a deficiency of nitrogen as the leaves did not show symptoms of nitrogen starvation, viz. premature yellowing. Application of the sulphate of ammonia did not produce any effect on seed maturity. The leaves drooped and were

shed where even nitrogen was applied. It is probable that the roots in the saline layers of the subsoil did not normally function and the absorption of water and salts was interfered with. The leaves showed low nitrogen and lime contents at all stages of growth and a drop in the protash content from the flowering stage.

Though nitrogen was found to be low in the leaves, no deficiency of nitrogen in the carpels of bolls was found to occur. Though there was an accumulation of nitrogen in the carpels the potash content was below normal. Thus tirak-affected bolls from both the soil types

showed a low potash content.

The deficiency of potash in the carpels of trrak-affected bolls was again confirmed by analysing the carpels of bolls from normal and tirak-affected plants. The choice of plants was perfectly random irrespective of soil conditions. The normal and tirak-affected plants were collected from different fields. The bolls from normal and tirak-affected plants were separately analysed.

TABLE III

Percentage of potash in the carpels of normal and tirak-affected bolls

	Number of samples analysed												
	1	2	3	4	5	6	7	8	9	10	11	12	Mean
Normal bolls	4.93	4.51	4.22	4 61	4.17	<b>3</b> ⋅76	4.87	4.84	4.38	4.33	4.22	4-45	4.44
Tirak-affected bolls	2.61	2.22	2.34	<b>2</b> ·06	2.37	2-85	2.94	2.81	2.75	2.83	2.86	3.04	2.64

(S. E. 0.095)

The potash content of the carpels of tirak-affectad bolls was found to be significantly lower than the potash content of the carpels of normal bolls. The difference between nitrogen contents of the carpels of normal and tirak-affected bolls were not constant. In some cases the nitrogen contents were higher and in other cases lower in tirak-affected bolls than the nitrogen contents of the normal bolls depending on the nature of the soil type from where the samples were collected.

The shift in the time of sowing of cotton from the month of May to the month of June has been found to be the best remedy for tirak on soil with saline subsoil. The delay in sowing is accompanied by a reduction in vegetative growth of the crop which does not suffer from

a condition of physiological drought on such soils. The leaves do not droop and the seeds properly mature in the bolls[Dastur and Mukhtar Singh, 1942]. The ameliorative effect on tirak of June-sowing was found to be accompanied by a normal uptake of nutrients. The absence of drooping itself indicated normal absorption of moisture. The leaves of the May-sown and the June-sown plants on normal soils and on soil with a saline subsoil were analysed at fortnightly intervals from the early stages up to maturity. for nitrogen, phosphoric acid, potash and lime (Figs. 14 and 15). The nitrogen, potash and lime contents of the leaves of the June-sown plants were higher at all stages of growth than those of the leaves of the May-sown plants on soil with saline subsoil. The results indicated that a

June-sown crop was also able to function normally on the soils with a saline subsoil, and was able to obtain its normal requirements of water and salts.

# SUMMARY

A study of the mineral uptake of normal plants and of tirak-affected plants on the two soil types (a) light sandy deficient in nitrogen and (b) soil with saline sub-soil was made to determine, if a deficiency of any important mineral was associated with immaturity of seeds in tirak-affected plants.

Detailed investigations of the chemical composition of leaves and bolls at different stages of development of normal and tirakaffected plants on the two soil types have revealed that a deficiency of potash occurred at the fruiting stage in tirak-affected plants. It was a common feature of tirak-affected plants occurring on both the soil types.

The deficiency of potash was found to occur indirectly as direct applications of potash were not found to increase its uptake or to increase seed maturity, The deficiency of potash in light sandy soils occurred as an indirect result of a deficiency of nitrogen. If nitrogen was applied the uptake of potash was also increased.

On saline soils the deficiency of potash probably developed as a result of the development of a condition of physiological drought. The absorption of water and nutrients was therefore reduced. If the condition of physiological drought was prevented in such soils by reducing the plant size by a shift in the time of sowing the uptake of nutrients of which potash was one was found to be normal-The leaves of the late-sown crop did not droop and the seeds and lint matured properly.

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# STUDIES IN THE PERIODIC PARTIAL FAILURES OF THE PUNJAB-AMERICAN COTTONS IN THE PUNJAB

XV. FORMATION OF PROTEINS, OIL AND CELLULOSE IN THE BOLLS OF NORMAL AND TIRAK-AFFECTED PLANTS \*

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(With five text-figures)

THE studies of the mineral uptake of tirakaffected plants indicated that a deficiency of potash on both types of soils was associated with the immaturity of seeds. On light sandy soils the deficiency of potash occurred indirectly as a result of deficiency of nitrogen while on saline subsoils it appeared to occur on account of the development of a condition of physiological drought which interfered with the normal absorption of water and other nutrients. Applications of nitrogen to light sandy soils were found to increase the uptake of potash and it was accompanied by a reduction in immaturity of seeds, while nitrogen application on soils with saline subsoils did not give

similar results.

A deficiency of potash or nitrogen had already been known to cause a depression in the synthesis of proteins in plants and an accumulation of soluble forms of organic and inorganic nitrogen. The soybean and the pumpkin plants when starved of potash were found by Burrell [1926] richer in soluble nitrogen especially amino nitrogen than the control plants. Engel [1929] found a similar accumulation of soluble forms of nitrogen and a reduction of both total and protein nitrogen when nitrogen was deficient. Philips, Smith and Dearborn [1934] working on the tomato plant confirmed the findings of Burrell [1926]. They reported an accumulation of degradation products of proteins in the later stages of plant growth when potash was deficient. Walls [1940] who investigated the role of potasium in plants reported that the potassium-deficient tomato plant accumulated ammonia, amide and amino nitrogen, and showed a decrease

in the protein nitrogen. Richards and Templeman [1936] and Richards [1938] working on barley leaves determined the changes produced in the protein and carbohydrate metabolism when nitrogen, potash, phosphoric acid and lime were not supplied in adequate amounts. They found that when potassium was deficient the protein nitrogen disappeared quickly from the leaves, a marked increase in the amino and amide nitrogen occurred and in the later stages nitrates accumulated. the case of nitrogen-starved plants a fall in the level of nitrogen occurred with a corresponding fall in ding fall in protein nitrogen. Gregory and Sen [1937] had concluded that potash deficiency caused an increase in the amino nitrogen, while a nitrogen deficiency produced a reduction in the protein nitrogen. Similar results as discussed above were obtained by Nightingale [1937]. An accumulation of amide, ammoniacal, and amino nitrogen and other related soluble compounds was found to occur when potash was deficient.

The above mentioned investigations clearly pointed to a disturbance in the protein metabolism of plants, when either potash or nitrogen was low. It was therefore considered necessary to study the protein metabolism of the bolls to determine if immaturity of seeds, the main symptoms of tirak, was in any way associated with a disturbance in the synthesis of proteins. The seeds from tirak-affected bolls were known to possess very thin and papery cotyledons devoid of oil. The cotyledons did not get filled up with oil when the seeds failed to mature. It therefore appeared probable that the synthesis of oil was also adversely affected. The study of oil formation and the stages at which oil formation in the developing bolls ceased in tirak-affected plants would provide information on the relationship of this process to the synthesis of proteins and

consequently to the growth of bolls. The

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determination of physical and chemical constants of oil in the normal and tirak-affected bolls was considered of importance to determine whether the nature of the oil produced was the same or not.

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This study of the fat metabolism of bolls would not be complete without a similar study of the carbohydrate contents in the developing bolls of normal and tirak-affected plants, as it was well known that oil was produced at the expense of carbohydrates. Caskey and Gallup [1931] have found that oil increased repidly in the cotton seed from the 21st day of the setting of the boll up to the 30th day while sugars decreased in all parts of the bolls as the development proceeded. These findings of Caskey and Gallup [1930] on cotton seeds agreed with those of Gerber [1897] on walnut and almond, of Ivanow [1911, 1912] on flax and rape seeds, of Rushkovski [1930] on sunflower seeds, of Eyre [1931] and of Johnson [1932] on the oil formation in the seeds of flax and of Sahasrabuddhe and Kale [1933] in niger seed. Reeves and Beasley [1935] who studied the development of the embryo of cotton seed found that sugars were present from the very beginning of the development of the embryo, while oil appeared during the 3rd week.

# MATERIALS AND METHODS

As described in the previous contribution [Dastur and Ahad, 1944], three types of soils were selected for this study, viz. (1) Normal sandy loam, (2) Sandy loam with saline subsoils, and (3) Light sandy soil deficient in nitrogen. About 5000 flowers were tagged at the end of September in each soil type. Weekly samples of boll material were taken early in the morning from tagged plants. The samples were brought to laboratory and immediately weighed.

After sampling, the bolls were divided into various parts, viz. carpels, seeds and lint. Fresh weights, percentage of moisture and dry weights were determined from a part of the sample which was used for the determination of total nitrogen. For drying the samples electric ovens were used. For the determination of soluble tractions of nitrogen fresh material was used and the following procedure was adopted.

The method of squeezing out the juice by pressure from the frozen plant materials commonly employed with leaves and other tender parts could not be adopted in this case, as the carpels contained fibrous tissues and the seeds became dry and hard as they developed. Due to these practical difficulties, the grinding

method of Davidson and Shive [1934] was finally adopted. About 25 to 50 gm. of the chopped fresh material was put in about 100 c.c. of boiling water to kill the enzymes. The material was then ground with water in a stone mortar and squeezed through muslin cloth in a beaker. This process of grinding and squeezing was repeated several times till all the soluble forms of nitrogen were removed. Two c.c. of 10 per cent acetic acid were added in the extract and it was boiled for one minute to precipitate any protein nitrogen left in the colloidal form in the solution. After cooling, the solution was made up to 500 c.c., a few drops of toluene were added and it was kept in a refrigerator for 48 hours at a temperature lower than 5° C. so that most of the colloidal particles may settle down after chilling. The clear liquid was then used for the analysis of various soluble forms of nitrogen. Adequate quantities from the extracts, which were kept in the refrigerator, were taken for each analysis.

Total nitrogen and soluble total nitrogen were determined by the Kjeldahl-salicylic acid method modified to include the nitrogen of of nitrates. Protein nitrogen was calculated by difference between the total nitrogen and the total soluble nitrogen. The nitrate nitro-gen was determined by the modified phenoldisulphonic acid colorimetric method adopted by Frear [1930]. Ammoniacal nitrogen was determined by magnesium oxide method modified by Schlenker [1932]. Amide nitrogen was determined by the method of Tottingham et al. [1935] and a amino nitrogen by Van Slyke's method [1932]. Phosphotungstic method of Leonard [1936] was used for determining diamino and basic nitrogen.

Oil was determined by extracting the ground material with petroleum ether and its melting point by method of Jamieson [1932]. Abbe's Refractometer was used for determining the refractive index. Iodine, acid and saponification values of oil were determined by standard methods.

The fresh material was killed by immersing it in boiling alcohol to kill the enzymes. The soluble forms of carbohydrates were extracted with 80 per cent alcohol in a Soxhlet's apparatus for 24-30 hr. Alcohol was distilled off under reduced pressure at 40°C. and residue was dissolved in warm water. Basic lead acetate and sodium biphosphate were used for clarification. The extracted material was dried, finally powdered and passed through 100 mesh sieve for the determination of starch.

Reducing sugars and total sugars were determined by the Schaffer-Somogyi Copper volumetric micro-method modified by Heinze and Murneek [1940]. Starch was determined by Starch-iodide gravimetric method modified by Chinoy [1938]. Purified lint free from fats and sugar was estimated by the stardard chlorination method [Doree, 1933]. a-cellulose was estimated by gravimetric method after treatment with 17.8 per cent sodium hydroxide according to the technique recommended by the Committee of the Division of the Cellulose Chemistry of the American Chemical Society [1929]. \( \beta \) and 8-cellulose were determined from the filtrate of a-cellulose by potassium chromate-ferrous ammonium sulphate volumetric method according to Bray and Andrews

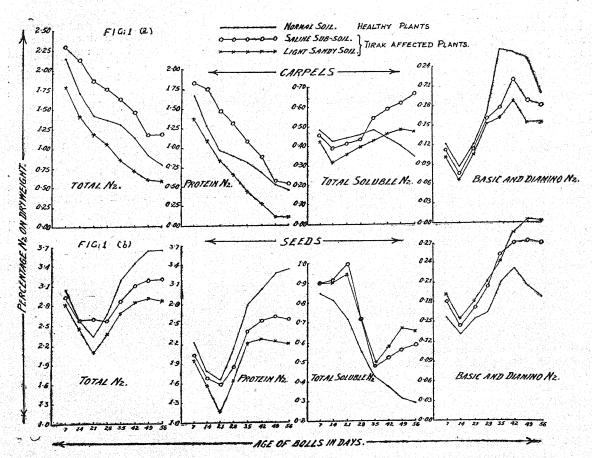
Investigation

Nitrogen metabolism

The trends in the total nitrogen and protein nitrogen contents in the carpels and seeds of bolls of normal plants and tirak-affected plants were similar (Fig. 1 a, b). The total nitrogen and protein nitrogen declined in the carpels as the bolls developed. The fall in the early stages may be due to a rapid increase in the dry weight of the carpels. There was a decrease in the total and protein nitrogen contents of the seeds up to the third week of boll development after which these contents increased in the seeds of normal as well as tirak-affected bolls (Fig. 1 a, b).

There were however marked differences in the concentration of total nitrogen and protein

[1923]. Percentage of total N, protein N, soluble N and diamino N in the (a) carpels and (b) seeds of normal and tirak-affected bolls



nitrogen at different stages of growth between normal and tirak-affected bolls. The total of bolls from saline subsoils were higher than

nitrogen and the protein nitrogen in the carpels

the similar contents in the carpels of normal bolls. Thus, nitrogen deficiency was not indicated in the carpels of the bolls from this type of soil. This difference already reported in the previous contribution [Dastur and Ahad, 1944] was, therefore, again confirmed. In the case of sandy soils total nitrogen and protein nitrogen in the carpels were found to be lower than those of the carpels of bolls from normal soil.

The total nitrogen and the protein nitrogen contents were highest in the seeds of normal bolls; medium in the seeds of tirak-affected bolls from saline subsoils; and lowest in the seeds of tirak-affected bolls from light sandy soils. The protein nitrogen did not show much increase in the seeds of tirak-affected bolls from the two soil types in the last three weeks of development. The synthesis of proteins in the seeds of tirak-affected bolls appeared to cease at the end of the fifth week.

The trends in the concentration of soluble nitrogen in the seeds and the carpels of bolls from the normal and tirak-affected plants were found to be quite different. In the carpels of bolls from normal plants the soluble nitrogen decreased from the 5th week, while it increased up to the last stage of development in the carpels of tirak-affected bolls (Fig 1 a, b). The soluble nitrogen in the seeds of normal bolls showed a continuous decline from the 1st week up to the 8th week of development (Fig. 1b) while it showed an increase in the seeds of-tirak affected bolls from the 5th week of development. The conversion of soluble nitrogen to protein nitrogen did not, therefore, occur in the seeds of tirak-affected bolls from the 5th week of development. The accumulation of soluble nitrogen in the bolls of tirak-affected plants began a week before the protein synthesis ceased. The protein metabolism of tirak-affected bolls was therefore not found to progress normally from the 5th week of development.

The curve showing the diamino nitrogen in the carpels and seeds of normal and tirak-affected bolls (Fig. 1 a, b) indicated that the synthesis of proteins in the tirak-affected bolls ceased at the diamino stage. The diamino nitrogen in the carpels of normal bolls was higher in the last four weeks of development than the same form of nitrogen in the carpels of bolls of tirak-affected plants, while reverse was the case in the seed. The soluble nitrogen in the carpels and seeds of normal bolls chiefly consisted of diamino nitrogen which appeared to be converted into proteins.

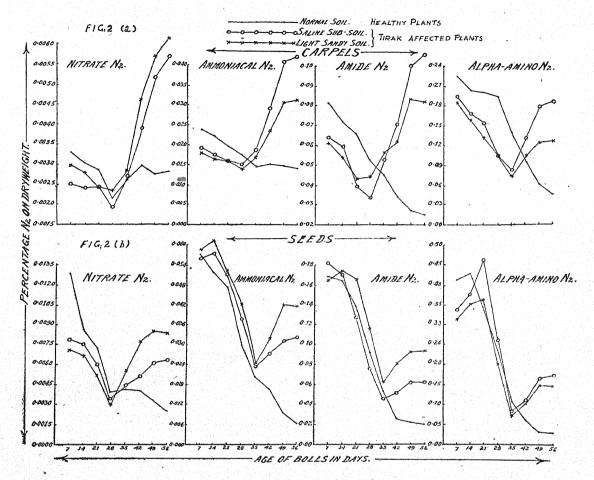
The other fractions of soluble nitrogen in the carpels and seeds of normal and tirak-affected bolls showed similar differences from the 5th week of development. Up to the 5th week the curves of nitrate ammoniacal, amide and amino nitrogen were of descending nature, showing that these compounds did not accumulate in the bolls and the protein metabolism proceeded normally in all bolls. But after the 5th week of development these soluble fractions began to accumulate in tirak-affected bolls (Fig. 2, a, b). Amide nitrogen and amino nitrogen showed a continuous decrease in the seeds and carpels of normal bolls, while they accumulated in the carpels and seeds of tirak affected bo Is from the 5th week of development. The accumulation of nitrate nitrogen was found to occur a week earlier (Fig. 2 a, b).

The above results suggested that accumulation of nitrate and ammoniacal nitrogen started in tirak-affected bolls during the 5th week of development. This was followed by an accumulation of soluble organic nitrogen during the 6th week and after. This was in turn accompanied by a cessation in the synthesis of proteins at the same stage in the seeds of tirak-affected bolls. The disturbance in the protein metabolism in the bolls of tirak-affected plants could, therefore, be said to occur, when the bolls were about five weeks old.

The total nitrogen and the protein nitrogen contents of turak-affected bolls from light sandy soils were lowest at all stages of development. This may be due to a deficiency of nitrogen and potash occurring together in the plants on this type of soil.

The lint of the bolls, from the three soil types were analysed for total nitrogen, protein nitrogen and soluble nitrogen. The total nitrogen in the lint decreased as the lint matured. This was also found to be the case in the carpels. There was, however, a temporary increase in the protein nitrogen in the first three weeks of development after which there was a decline. The temporary rise in the protein nitrogen in the lint in the first three weeks may be due to maximum production of new hairs during that period, after which formation of new hairs did not occur. As these newly formed hairs were living and filled with protoplasm, the protein nitrogen contents were also high. In the later stages the hairs elongated and thickened and most of the cell contents disappeared whereby a decrease in the percentage of total nitrogen, protein nitrogen and soluble nitrogen occurred.

Percentage of nitrate N, ammon acal N, amide N and alpha-amino N in (a) carpels and (b) seeds of normal and tirak-affected boils



Fat and carbohydrate metabolism

The results of oil contents and the different constants of oil are given in Table I. The ether extract of the seeds in the first three weeks of development consisted of a waxy substance of a high melting point fluctuating between 52°C. to 55°C. The real oil formation started from the 4th week of boll development in the case of normal plants and of tirak-affected plants on light sandy soils while oil appeared a week earlier in the seeds of tirak-affected plants on soils with saline subsoil. The oil formation ceased in the 6th week in seeds of tirak-affected plants while it continued to increase up to the 8th week in normal plants, i.e. when the bolls opened. The quantity of oil formed per 100 gm. of seeds in tirak-affected plants was less than in the seeds of normal plants.

The melting point of the ether extract was gradually lowered as the seeds developed till it reached a value of 2°C. to 3°C. at maturity in seeds from normal as well as tirak-affected plants. There was an increase in the saponification and iodine values of the ether extract as the seeds matured indicating that the fatty acids of low molecular weights and the unsaturated fatty acids in the oil increased as the seeds developed. The values of these constants of ether extract were slightly lower in the seeds of tirak-affected plants than in the seeds of normal plants. The acid value of the extract was found to decrease as the seeds developed indicating a decrease in the free fatty acids. The ether extract of the seeds of tirak-affected plants showed slightly higher amounts of free fatty acids than the ether extract of seeds of normal plants.

Table I

Analysis of the oil from the seeds at different stages of growth

	Age	e of bolls		Per cent oil	Iodine value	Saponification value	Acid value	Melting point °C.	Refractive index	
					(a) Seeds	from normal soil				
7	days			1:33	36.45	155-20	56.40	52.0	1.485	
4	days		•	1.31	34.86	158-80	57.05	50·5	1.485	
1	days		•	1.34	37 05	157-60	55·92 <b>*</b>	51.0	1.485	
8	days		••	6.16	85.70	179-30	31.37	7.0	1.475	
5	days			15.23	96.22	183-20	18-64	3.5	1.474	
2	days			21.17	102.50	189.50	8-74	2.2	1.474	
9	days			23 96	108.40	193-40	2.55	2.0	, 1.474	
в	days			24.59	109.55	194.70	0 45	2:1	1.473	
					(b) Seeds f	rom saline soil				
7	days			• 1·33	37-81	[ 151.80 ]	54·02	51.0	1.486	
	đays			1.36	35.31	156-60	52.42	51.5	1.485	
	days		••	4.81	62.88	170-50	38-93	10 0	1.476	
	days	•		9.61	83-38	177-10	29.16	9.0	1.475	
	days			11.81	92-25	182-50	17:48	3.5	1.474	
2	days			12.38	96.30	185-70	10-51	2.8	1:474	
9	days			12:41	101.75	186-20	7.64	2.5	1.474	
6	days			12.67	101-20	187-80	5.45	2.4	1:474	
					(e) Seeds	from sandy soil				
7	days			1.28	36-65	153-80	54.43 (	50•4	1.486	
4	days			1:35	35·27	158-50	55.40	51-0	1.485	
	days			1:31	38-72	157-30	54.15	50-0	1.485	
8	days			6.01	82-62	174-60	32 28	10.0	1:475	
5	days			9.42	91-26	182-40	19-11	4.0	1:475	
2	days			9.98	95•50	183-10	11 64	2:5	1.474	
9	days		*	10:34	100-82	186-60	8-65	2:3	1:474	
6	days			10.18	100-20	188-40	6 86	2.2	1.474*	

Thus except for minor differences in the formed in normal and tirak-affected plants different constants of oil, the nature of the oil appeared to be the same.

The reducing sugars, disaccharides, starch, and oil contents of the seeds from normal and tirak-affected plants are given in Fig. 3. The most noticeable feature was the fall in carbohydrates and a rise in oil content as the seeds matured. The reducing sugars and starch were present in larger amounts than disaccharides

which were present in very small amounts.

The carbohydrate analysis of the carpels (Fig. 4)showed that reducing sugars were present in largest amounts. They showed a rise in the first two weeks after which they declined rapidly. The starch and disaccharides showed a decline at later stage.

Fig. 3. Percentage carbohydrates and oil in the 4F developing seeds of normal and tirak-affected plants

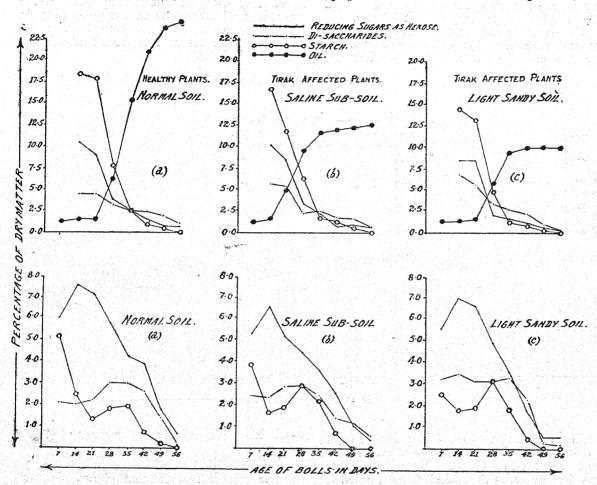


Fig. 4. Percentage carbohydrates in the developing carpels of normal and tirak-affected 4 F plants

Carbohydrates of lint

The lint from bolls of tirak-affected plants is known to be weak. The fibres easily break and consequently they are considered immature. As fibre strength is an economically important character, chemical nature of lint from normal and tirak-affected plants was determind.

Hawkins and Serviss [1930] studied the development of cotton fibre in the Pima and Acala varieties of cotton and found that the elongation of the fibre was nearly complete in the first three to four weeks of boll development and

the thickening of the fibre occurred during the remaining period. The development of lint in relation to soil coditions was studied by Sturkie [1934] and he concluded that the soil moisture was an important factor that influenced the maturity of fibres in cotton plant. When the available soil moisture was low, it produced a weak and short fibre. Hawkins [1931] also found that high concentration of soil alkali and comparatively low supply of soil moisture produced high percentage of immature seeds and fibres. These findings are in conformity with the

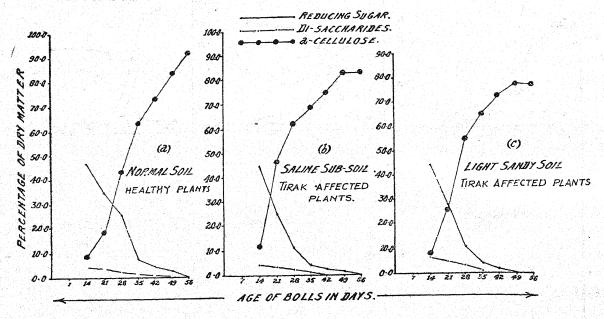
findings of this investigation as on soil with saline subsoils tirak occurred on account of unavailability of moisture. The biochemistry of cotton fibre at different stages of growth had also been studied by Jack and Forest [1940]. They found that the young fibre contained large quantities of reducing sugars relative to the mass of the crude fibre and there was a progressive reduction in the reducing sugars during the period when the fibres began to grow in thickness.

The structure and constitution of cotton fibre has also been investigated by various workers like Denham [1923], Kerr [1937], Kerr and

Anderson [1938] and Nickerson [1940].

The carbohydrate analysis of the lint showed that reducing sugars were present in largest quantities in early stages. Fifty per cent of the dry weight of lint in the second week of development consisted of reducing sugars while disaccharides were present only in very small amounts. There was a progressive decline in reducing sugars of lint as it matured and it was almost nil in the fully mature fibres (Fig. 5). No differences were found between the carbohydrate contents of lint from trak-affected and normal plants.

Fig. 5. Percentage carbohydrates and alpha cellulose in the 4F normal and tirak-affected developing lint



The progressive decline in reducing sugars was accompanied by an increase in the cellulose content of the lint (Fig. 5 a, b, c). Thus there was clear evidence that callulose was formed at the expense of reducing sugars. The molecules of cellulose are known to consist of a number of anhydrous glucose molecules and its synthesis from reducing sugars in the case of lint can be regarded as highly probable.

Further analysis of cellulose of the lint revealed that it was mostly composed of α-cellulose. β-cellulose and 8-cellulose were found in very small qunatities. The lint of normal plants contained about 91 per cent of α-cellulose on dry weight basis, while the lint of tirak-affected plants from soils with saline subsoil and light sandy soils contained 82 and 76 per cent of

a-cellulose respectively at maturity (Fig. 5 a, b, c).

Technological study of lint

The lint of tirak-affected plants, although weak in strength, is not reduced in length. The decrease in the cellulose contents of the lint in tirak-affected plants may, therefore, be due to a decrease in its growth in thickness as the secondary thickening of the fibres generally occur as a result of deposition of fresh cellulose layers on the primary cell wall. The cellulose content of the lint from tirak-affected plants was found to be lower than that from normal plants. This conclusion was further supported by the technological tests carried out on the lint from normal and tirak-affected plants at the laboratory of the Cotton Botanist, Mirpurkhas.

Table II

Technological properties of lint from normal and tirak-affected plants

	Mean fibre	Mean length	Matu	Maturity count (per cent)				
Lint samples collected from	weight 10-6 gm.	in inches	Mature fibres	Half mature fibres	Immature fibres			
1. Normal soil	0.172	0.79	76	10	14			
2. Saline sub soil	0.128	0.71	48	12	40			
3. Light sandy soil	0.114	0.71	38	18	44			

There was no appreciable difference in lint length, but there was marked difference in the fibre weight per unit length. The fibre from normal plants weighed more per unit length than the fibre from tirak-affected plants. Thus the conclusion that the lint of tirak-affected plants did not grow in thickness by secondary deposition of cellulose to the same extent as it did in the case of normal plants was again confirmed. This was also clear from the maturity counts given in the same Table. The lint of tirak-affected plants was found to contain a higher proportion of immature fibres than the lint of normal plants.

## Conclusions

The protein metabolism of the bolls of tirakaffected plants appeared to deviate from the
normal course from the fifth week and the
synthesis of proteins in the seeds did not occur
in the former in the last three weeks of development

There was an increase in protein nitrogen and a decrease in soluble nitrogen as the seeds developed indicating that protein nitrogen was synthesized from the soluble forms of organic nitrogen. The primary synthesis of proteins generally occurs in the leaves. The proteins hydrolize afterwards into soluble forms of organic nitrogen and travel to different parts of the plants. In this case they travelled from the leaves to the developing bolls. This conclusion was also supported by the fact that percentage distribution of total nitrogen declined in the leaves at the fruiting stage while it increased in the bolls [Dastur and Ahad, 1941].

The concentration of potash was also found to decrease in the leaves at the fruiting stage and in the carpels from the fifth week of develop-

ment in tirak-affected plants while it continued to rise in the carpels of normal bolls. Thus lack of potash probably appeared to be related to the cessation of protein synthesis in tirakaffected plants and whatever small amounts of soluble forms of organic nitrogen reached the bolls they remained unconverted into proteins and consequently accumulation of soluble nitrogen occurred in the carpels and seeds, mostly in the forms of amides and amino acids. Richards and Templeman [1936] pointed out that potassium in some manner was essential for the maintenance of the protoplasmic complex. and in its absence protoplasm did not function normally, and consequently further protein synthesis was checked and accumulation of simpler nitrogen fractions occurred throughout the plant. The results discussed above showed somewhat similar trends.

It was already shown [Dasur and Ahad, 1944] that the bolls from tirak-affecteed plants did not increase in dry weight from the fifth week of development, while in the case of normal boils the increase in the dry weight continued up to the last stage of development. Thus when protein synthesis stopped the growth of tirak affected bolls had also ceased.

The results described above definitely indicated that proteins and oil in seeds and cellulose in lint were formed from carbohydrates. The reducing sugars appeared to be the main carbohydrate utilized in the formation of protein and oil in seeds and of cellulose of lint as this kind of sugar declined in carpels, seed and lint as development proceeded. The starch and disaccharides found in the carpels and seeds may be regarded as temporary storage products resynthesized from reducing sugars. These higher forms of carbohydrates appeared to be reconverted into reducing sugars as the latter

were being utilized in synthesis of protein and

The cellulose content of the lint from normal plants was higher than that of the lint from tirak-affected plants and that was due to a decrease in the secondary thickening of the fibre in the latter. Consequently the lint from tirak-affected plants was weak in strength.

# SUMMARY

The fromation of proteins in bolls of tirakaffected cotton plants ceased from the fifth week of development while proteins continued to be synthesized up to the eighth week of boll development in the normal plants. The cessation in protein synthesis in bolls of tirak-affected plants was accompanied by an accumulation of soluble froms of non-protein nitrogen from the same stage of development. These froms of nitrogen, on the other hand, were found to decline continuously in the bolls of normal plants. A disturbance in the synthesis of proteins in the bolls of tirak-affected plants was therefore clearly indicated and the accumulation of soluble forms of nitrogen could be linked up with a deficiency of potash in the leaves and carpels. The decreased rate of protein formation in bolls of tirak-affected plants was associated with a decrease in dry matter of the bolls. Thus the growth of bolls ceased at an earlier stage in the tirak-affected plants as compared with normal plants.

The corbohydrate analysis of bolls did not show any differences between the normal and tirak-affected plants though a decrease in the oil content was found to be a feature of the bolls of the latter. Thus carbohydrate supply did not appear to be responsible for either decreased protein or oil synthesis in the bolls of tirak-affected plants. Potash deficiency [Dastur and Ahad, 1944], therefore, appeared to be the primary cause that led to the development of

immature seeds in tirak-affected plants.

The anslysis of lint showed that cellulose was formed at the expenses of reducing sugars. The main differences between normal and tirakaffected bolls were found to be in the cellulose content of the lint, the percentage of the cellulose being much higher in the lint of the former than in the lint of the latter. The decreased cellulose content appears to be associated with a decrease in the secondary thickening of the fibre as the lint length was found to be nearly the same in the normal as well as tirak-affected plants.

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# STUDIES ON STORED GRAIN PESTS IN THE PUNJAB VI. BIOLOGY OF TROGODERMA GRANARIUM EVERTS\*

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(With one text-figure)

NINETEEN insects have so far been recorded as infesting food grains in the Punjab [Rahman, 1942] and out of these 'Khapra' (Trogoderma granarium Everts: T. Khapra Arr.) is the most abundant and destructive in our commercial centres of wheat production. Barnes and Grove [1916] had worked out the biology of this pest in broad outlines only. In order to subjugate a pest completely and cheaply, a thorough knowledge of its biology is essential. Hence

observations on the biology of this important pest were taken up seriously in 1939 and the results are presented in this paper.

# DISTRIBUTION

Trogoderma granarium Everts is a native of India. It has been carried to the following countries mostly on wheat and barley: Britain, Germany, Holland, Korea, Japan, Odessa, North America and Rotterdom. In India, it is found in U. P., Central Provinces and Berar, Sind, North West Frontier Province, Gwaliar and the Punjab. In the Punjab, it is confined to the plains where it causes the greatest damage to wheat particularly in the hotter and drier parts, the areas of its greatest destructiveness being the canal colonies [Rahman, 1942].

## FOOD

T. granarium is a very serious pest af wheat. In addition to the food mentioned by Rahman [1942], it has also been recorded from brain in

<sup>\*</sup>I. Observations on the reactions of Dermestid beetle, Troqoderma Khapra Arr. to light. Indian

J. Ent. 1, 57-63 (1939)

II. Insect pests of stored grains in the Punjab and their control. Indian J. agric. Sci. 12, 564-87 (1942)

III. Biology of Bruchus analis Fab. and B. chinensis Linn. (Bruchidae: Coleoptera.) in the Punjah. Indian J. agric. Sci. 12, 851-64 (1942)

grain from insects. Indian Fmg. IV. Save stored

<sup>4, 18-20 (1943)</sup>V. Fighting 'Khapra' in the Punjab. Indian Fmg. 5, 272-5 (1944)

the Punjab, malt in Germany and England, pulses, oats and rye in Germany. Wheat, however, is its principal and most favoured food.

#### LIFE HISTORY

Precogulation period. According to Nakayama [1933], the adults mate 2-3 days after emergence; we, however, found them to mate immediately after emergence.

Preoviposition period. According to Voelkel [1924] a female started laying eggs 5-6 days (at 25°C.) after mating while according to Nakayama [1933] it oviposited immediately after mating. We found the duration of the preoviposition period to depend upon the time of the year: it lasted for 2-4 days in April, 1-2 days during May-September and 2-5 days in October.

Oviposition. Barnes and Grove [1916] recorded the adults and the eggs during April-September, but we have found that the females from the over-wintered grubs started egg laying in the second week of April and their succeeding generations continue to oviposit up to the end of October in the laboratory and up to the end of November in the heavily infested stores. According to Barnes and Grove [1916]

and Mason [1921], the eggs are laid in the groove or some other portion of the surface of grain, while Rahman [1942] and others assert that they are laid among the grain generally singly, rarely 2-5 together. Barnes and Grove [1916], Voelkel [1924], Mason [1921], Morison [1925] and Nakayama [1932] maintained that a single female laid 35-40, 65 at 30°C., 35-40, 50 and 20-30 eggs respectively in her life time, while Rahman [1942] stated that in its life time a female laid 85 eggs in 7 days at the rate of 1-26 eggs per day. During our present investigations, however, we have found that there is a considerable variation in the amount of oviposition among the individual females in all the months and a female has been observed to lay 4-89 eggs in 1-8 days at the rate of 1-55 eggs per day. Maximum amount of oviposition recorded during these investigations was about double, i.e. 89 as compared to that recorded by Barnes and Grove [1916], i.e. 41. Voelkel [1924], recorded 126 to be the maximum number of eggs laid by a single female at 30°C. A female laid the highest number of eggs during April-May and least during October. Table I gives the oviposition period, total number of eggs laid by a female and its daily rate of oviposition for each month from April to October.

Table I

Oviposition record of T granarium Everts

Month	Total	Number o laid	f eggs	No	o. of eggs land	aid	Oviposition period (in days)		
	Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver
April	89	7	52.2	55	1	8.7	8	2	6.0
May	88	13	52.7	44	2	12.0	7	2	4.4
une	 85	13	30.4	22	1	7.5	6	2	4.1
uly	47	4	23.7	18	1	6.8	5	1	3.5
ugust	 60	12	32.0	26	2	7.8	7	1	4.1
eptember	 52	9	31.0	24	- 1	5.7	6	2 2	4.2
October	28	4	15.9	9	1	4,5	. 5	2	3.6

Egg. It measures 0.11 mm in length and 0.03 mm in its greatest diameter.

Incubation period. Barnes and Grove [1916], Mason [1921] Morison [1925], Nakayama [1932] and Rahman [1942] have stated that the egg stage occupied 5-7, 6-7, 5-9at 30°C, 6-12 and 5-10 days respectively. We have found the incubation period to vary with the season; eggs laid in April hatched in 5-9 days, those laid during

May-August hatched in 3-6 days while those oviposited in September and October took 5-10 days to hatch (Table II). This shows that contrary to the observations made by Barnes and Grove [1916], there is an extensive variation in the duration of the egg stage during different parts of the year, i. e. in June egg stage occupied about double the period (Table II) than that taken by it in April and October.

TABLE II Incubation period of T. granrium Everts

Month	No. of observa-	Incubation period (in days)					
	tions	Max.	Min.	Aver.			
April	180	9	5	7.1			
	477	6	4	5.4			
June July August	140	5	3	4.4			
	192	6	4	4.6			
	366	6	3	4.8			
September October	264	8	5	6.2			
	68	10	6	6.7			
				]			

Viability of eggs. Viability of the eggs was found to be 48.6 per cent in April, 41.5 per cent in June, 60.3-66 per cent in May and August, 51.95-58.4 per cent in July and October and 86.8 per cent (i.e. highest) in September.

Larval stage. Nakayama [1932] has recorded the larvel stage to occupy 317-351 days while according to Rahman [1942] the male and female larvae are full-fed in 19-28 and 20-37 days respectively. During our present investigations we found a male larva to be full-fed in 16-53 and a female larva in 20-63 days (Table III) and during this period they moulted 3-4 and 4-6 times respectively as against 4 and 6 recorded by Barnes and Grove [1916] respectively.

TABLE III Duration of the larvel stage of T. granarium Everts

	[14] 라마스 (14] 유민(지 14] 보호 (15]		Male	larvae	Female larvae					
N	Month ril-Mny ne ly gust	Number of	of					Duration in days		
		observa- tions	Max.	Min.	Aver.	observa- tions	Max.	Min.	Aver.	
April-May		47	53	16	37.9 24.0	59 36	63 43	25 23	40.9 27.8	
		1 26	34	18	21.5	33	$\frac{45}{37}$	20	26,8	

Overwintering larvae. Time of their entering into hibernation depended upon whether the larvae were feeding in the laboratory or in the godown. Those feeding in the laboratory started entering hibernation on 9 August while in the case of those in the godown hibernation began in the end of November. A few typical cases of the duration of overwintered larvae are given in Table IV.

TABLE IV Duration of the overwintered larvae

Date of hatching	Date of pupation	Duration in days
9 August 1941	3 May 1942	267
16 August 1941	30 May 1942	287
30 August 1941	9 April 1942	222
22 September 1941-	18 April 1942	208
1 October 1941	13 April 1942	194
15 October 1941	7 May 1942	214
16 October 1941	5 June 1942	171
26 October 1941	1 July 1942	186

Pupal stage. According to Mason [1921],

the larvae pupated inside the malt grain (which it never leaves until it eats up all the endosperm), while according to Rahman [1942] pupation takes place in the last larval skin among the grains. We found the pupae to abound usually in the top layers of the stored wheat. According to Nakayama [1932], the pupal stage was completed in 6-17 days, while according to Rahman [1942] it occupied 4-6 days. We, however, found the pupal stage to occupy 3-6 and 3-8 days in case of male and female pupae respectively.

Longevity of the adults. According to Barnes and Grove [1916] and Voelkel [1924], the adults before emergence remain in the last larval skin for 2-3 days and from several hours to 10 days respectively. We, however, found this period to last for 15-78 hours. Barnes and Grove [1916] have only recorded the maximum longevity of the adults (10 days) whereas Mason [1921] has found the adults to live from a few to 10 days. We have worked out the longevity of the female and male adults separately in different months of the year which is presented in

TABLE V Longevity of adults in different months

	Month	Month		Mules (in days)			Females (in days)			
			Max.	Min.	Aver.	Max.	Min.	Aver.		
April May June			14 10 6	3 4 3	10.9 7.2 4.9	13 15 14	7 5 5	10.8 7.2 7.4		
Tuly August September			7 10	4 4 4	5.4 6.4 8.0	7 14 11	4 5 5	5.7 7.2 8.2		
etober)			ii ii	5	8.9	13	7	9.8		

September.

It will be observed from Table V that both the males and females live the longest during April and shortest during June and July respectively.

> SEASONAL HISTORY AND NUMBER OF GENERATIONS

According to Barnes and Grove [1916], the pest remains active from April to September and in a completely dorment condition from Octotober to March. We, however, have found that the pest remains active from mid-March to October in the laboratory and up to the end of November or even later in godowns, where wheat is stocked in bulk and the infestation is very high. Further, it was observed in the laboratory that some of the larvae which hatched from the eggs laid on 9 August 1941 entered into hibernation, while the remaining larvae completed their growth and emerged as adults by September. This went on up to the 15 August 1941, after which date all the larvae which hatched out of the eggs entered into hibernation. Thus in the laboratory T. granarium started hibernation as larvae from 15 August. The seasonal activity of the pest is given below.

Mid April to end of Pupation starts. Adults April.

May to August.

January to mid-March. Only hibernating larvae present. Activity nil. Mid March to mid-April. Larvae resume activity. appear and lay eggs. Larvae and pupae also present.

All stages of the pest present, damage maximum In the laboratory some of the larvae hatching in the first

week of August enter into hibernation whereas all those which hatched after 15 August hibernated.

All stages present but pupae and adults comparatively less. Pest activity shows distinct decline.

Larvae preponderate October. over other stages. Activity greatly reduced.

Only larvae present. November-December. Activity particularly at stand still.

According to Barnes and Grove [1916] and Nakayama [1933], T. granarium has four and two distinct generations a year respectively, but we have found the pest to pass through 4.5 overlapping generations during the year (Fig. 1).

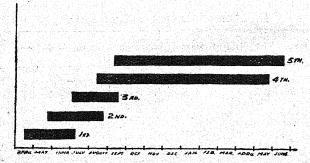


FIG. 1. NUMBER OF GENERATIONS OF I granarium EVERTS.

SEX BATIO IN DIFFERENT GENERATIONS

The ratio of the males and the females in different generations was almost at par except in the fifth generation, when the females predominated (Table VI).

VIABILITY OF DIFFFRENT STAGES

Viability of eggs was usually low in all the

generations except fourth, when it was 868 per cent, Viability of the larvae was fairly high throughout their period of activity. Mortality among pupae was negligible. Results of our observations on this point are presented in Table VII.

TABLE VI

Sex ratio of T. granarium Everts in different generations

		The Control of the Co			Sex	ratio
No. of g	generations	Total No. of adults counted	No. of males	No. of females	Male	Female
		245	119	126	48-2	51.8
lst		기가 가는 사람이 모르는 얼마에 모르는다.	53	50	51.3	48.7
2nd			75	70	51.7	48-3
Brd			117	127	48.0	52-0
4th		. 244	16	24	40.0	60.0
5th		. 40	1			

TABLE VII

Viability of different stages of T. granarium Everts in different generations

Generations	Eggs laid	Eggs hatched	Larvae pupated	Adults emerged	Percentage of viability			
		364	335	305	51.5	92.0	91.3	
st	713	140	110	107	41.5	78-6	97.3	
end ···	337	192	149	145	51•9	77-6	97.9	
3rd ··	370	. 064	244	244	86-8	92.4	100.0	
4th 5th	77	45	40	40	58-5	88-8	100 (	

# DEVELOPMENT OF LARVAE ON VARIOUS FOODS

4 . . . . . . . . . . . . . . . . P Development of the pest was studied during June 1943 on the following nine food stuffs: Wheat, barley, bajra (Penuisetum typhoideum), jowar (Andropogon sorghum), rice, pista (Pistacea vera), gram, and walnut. Twenty-six newly hatched larvae were confined on each food stuff in small petri dishes and the developmental periods of the male and female larvae together with their viability were noted. Results are tabulated below:

It will be observed from Table VIII that the larval duration was shorter on wheat, bajra, maize, jowar, and rice, and comparatively longer on barley and gram and longest on pista and walnut, while percentage of viability was highest on rice and lowest on jowar.

Amount of food consumed by the Larvae

A single wheat grain of known weight was given to each newly hatched larva in a petri dish and as soon as it changed into a pupa, the

grain was weighed again and the total and daily amount of food consumed by the female and male larvae was worked out seperately. Results are given in Table IX.

TABLE VIII

Comparative rate of development and the percentage of larvae successfully completing their growth on different foods

			Durati	on of the larv	al stage (in	days)		Percentage of
Food st	tuff		Male larva	e		Female larv	ae	viability of the larvae
		Max.	Min.	Aver.	Max.	Min.	Aver.	
Wheat		42	17	26.0	47	26	35.2	73.0
Barley		49	22	32.8	51	28	40.1	80.7
Gram	• •	40	34	35.8	52	32	42.0	61.5
Bajra		28	21	24.8	34	26	29.4	61.5
Maize		38	21	25.6	46	26	39.9	76.9
Jowar		31	22	25.0	34	24	29.2	34.6
Rice		33	22	28.0	46	24 26	37.0	88.4
Pista		50	34	38.4	52		43.5	57.6
Walnut		45	31	37.1	52	33 34	43.1	57.6

TABLE IX

Amount in mg. of food consumed by the larvae

			Male	larva			Female larva						
Month	Daily amount of food consumed				Total food consumed			Daily amount of food consumed			Total food consumed		
	Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.	
June ,.	0.444	0.074	0.173	5.0	1.5	3.3	0.561	0:136	0.401	14.8	5.0	10.0	
July	0-600	0.083	0.293	10.0	1.5	5.1	0.770	0.269	0.520	17.0	7-4	11 7	

It will be observed from Table IX that the female larva consumed nearly double the quanity of food as compared to the male larvae.

#### PENETRATION IN A HEAP OF WHEAT

Barnes and Grove [1916] observed that the insect mostly confines itself to the top 10-12 in. layer of grain in a heap but we have observed the insect to be almost equally serious up to 2.5 ft. depth from the top as given in Table X.

Table X

Percentage of attack in the upper 2½ ft. column of grain

Column of grain from the top	Percentage of attack
0-6 in	59·6 52·4 58·0 54·0 54·0

As we go deeper into the heap the attack goes on adiminishing and at about 6 ft. it is negligible. Along the walls and in the corners the insect may penetrate as deep as 9 ft.

# HEATING OF THE GRAIN

Presence of T. granarium among the grain causes heating. In a laboratory experiment conducted during June, July and early August, it was observed that the presence of 4000 larvae in a 5000 c.c. bottle full of wheat raised the temperature of the grain by 2.4°F. over and above the room temperatute and by 11.5°F. over and above the healthy wheat in a similar bottle. It was also noticed that at the time of pupation and emergence of adults, heating was not appreciable. So the extent of heating naturally depends upon the population of insect particularly the larvae. Observations carried out in big stores at different centres in the province, besides confirming the above finding, brought to light another important fact. As a result of this heating, the period of activity of this pest was extended and the pest was found co be actively feeding and breeding even up to the end of November since temperature conditions in the godown were quite favourable for its multiplication, i.e. 83°-105°F.

This shows that heating of wheat is an index of 'Khapra' activity and wheat in the godown that is hot to touch should be rejected. This important observation should be made use of by all Government Departments which are required to handle wheat while making purchases. The stockists on the other hand should take care to keep the godowns as cool as possible by providing proper circulation of air in them

#### EXTENT OF DAMAGE

Extent of damage is calculated on entirely different basis by the seedsmen and the merchants. To a seedsman, percentage of damaged grain in a sample is the true index of the extent of damage whereas to the general trade, the total amount of loss in weight as a result of insect attack matters the most. Observations so far carried out in different parts of the province have shown that the extent of damage caused by this insect depends upon (i) initial infestation of the store; (ii) infestation in the adjoining store; (iii) condition of stock to be stored; (iv) time of the year when the grain is stocked; (v) type of storage receptacle; (vi) method of storage; (vii) extent of ventilation in the store; and (viii) period of storage. Data so

far collected from the point of view of seedsmen and the merchants are given below.

# (a) From the point of view of seedsmen

In a single storing season T. granarium, on an average, has been observed to damage the grain to the extent of 5.9 to 32.8 per cent with a maximum of 73.0 per cent.

# (b) From the point of view of trade

In order to judge the destructive potentialities of 'khapra' alone, some stores were selected in which wheat was attacked by this insect only and was stored from July to November. Results are given in Table XI.

Table XI

Percentage of loss in weight in a single storing-season

Amount of wheat stored	Amount of wheat recovered	Percentage of loss in weight
2,000 maunds	1,955 maunds	2:25
365 ,,	345 "	5-47
805 "	775 ,,	3.72

# SUMMARY

Trogoderma granarium Everts. is a serious pest of stored wheat and is distributed all over Northern India. It is active from mid-March to October, but in the heavily infested godowns where the temperature rises due to insect activity, it has been observed breeding even up to the end of November.

A female lays 489 eggs in 1-8 days at the rate of 1-55 eggs per day. Incubation period lasts for 3-10 days, larval stage occupies 16-53 days in case of male larvae and 20-63 days in case of female larvae, pupal stage being completed in 3-8 days and the adults live for 3-15 days depending upon the season. It passes through 4-5 generations in a year.

Sex ratio and viability of different stages in different generations is given. Comparative development of the insect has been studied on new different foods. Larval duration is found to be shorter on wheat, bajra, maize, jowar, and rice and the percentage of the viability of the larvae is highest on rice and lowest on jowar, The daily consumption of food by a female larva varies from 0.136 mg. to 0.77 mg. which is double than that of the male larva. Incidence

of the pest at different depths in a heap is discussed. In a single storing season, the extent of damage caused by this insect from the point of view of seedsmen and trade has been worked out at 5'9-32'5 per cent and 2'25-5'47 per cent respectively.

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# COLLAR ROT OF PIGEON-PEA CAUSED BY PYTHIUM APHANIDERMATUM (EDSON) FITZ.

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(Received for publication on 13 September 1944)

IN July 1943, a few wilted plants of pigeonpea (Cajanus cajan) variety U.P. 132, were received for examination from the Agricultural Section of the Imperial Agricultural Research Institute, New Delhi. Transverse sections Institute, New Delhi. revealed the presence of fungal hyphae, especially in the xylem vessels. Isolations yielded a species of Pythium, besides Macrophomina phaseoli (Maubl.) Ashby, and a Fusarium Sp. different from Fusarium udum Butler, which causes the Fusarium wilt of this crop.

Isolat ons were again tried from speciemens collected from the same plot in August and September and these largely yielded Pythium Sp. Some of the isolations from plants collected in September, yielded Corticium Rolfsii (Sacc.)

The Pythium when grown on potato dextrose

agar produced abundant mycelium measuring 2.4-8.5h diam. It formed lobulate sporangia and swollen antheridia. The oogonia measured 21 (14-34)  $\mu$  and the oospores 17 (12-29)  $\mu$ . The fungus agrees closely with *Pythium Butleri* Subr. and P. aphanidermatum (Eds.) Fitz. These two fungi were considered by Carpenter [1921] to be identical and Mitra and Subramaniam [1928] regarded P. Butleri as a 'strain' of P. aphanidermatum producing different symptoms of foot rot on papaya and having larger oogonia and oospores than the typical forms. Drechsler [1934] who studied nearly 100 isolates from various plants considered that there are two distinct species, P. Butleri having somewhat larger oogonia and oospores than P. aphani dermatum. The measurements given by the several workers are as follows:

Species	Oogonia Oospores		Authority		
P. Butleri Subr.  P. aphanidermatum (Eds.) Fitz.  """  Pigeon-pea isolate	26 (18-33) µ 27 2-272 19.8-28.6 22 21 (14-34)	21 (13.5-25.3) u 22.5 17-19 14.3-20.9 17.5 17 (12-28)	Subramaniam [1919] Drechsler [1934] Edson [1915] Mitra and Subramaniam [1928] Drechsler [1934] Author		

It will be noted that the pigeon-pea isolate has average measurements agreeing closely with P. aphanidermatum, but the range agrees with P. Butleri. Drechsler [1934] gives no detailed statistical analysis and one is led to believe that the differences in average measurements may not be significant. Whether in the broader

meaning of Carpenter [1921], Mitra and Subramaniam [1928], or in the narrower sense of Drechsler [1934], however, the pigeon pea fungus must be regarded as Pythium aphanidermatum (Eds.) Fitz.

Experiments to test the pathogenicity of the fungus were made. In the pot experiments,

germination failed when the fungus was applied at the sowing time but when applied to the stem just above soil level, a few days after sowing, it caused a drying of the leaves and the tender shoots, and some plants dried up at the collar region. The fungus was reisolated from these plants.

Observations in the pots from where diseased specimens were obtained, showed that scattered plants had wilted over a large are, growing at a low-lying place in wet soil. The soil is such that it retains water at the upper

surface for a considerable time.

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# A NOTE ON THE DETERMINATION OF ORGANIC NITROGEN IN SOILS

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(Received for publication on 17 June 1944)

THE determination of organic nitrogen in soils is of great importance in all studies on the nitrogen status of soils. The method commonly used is to digest the soil with strong sulphuric acid in the presence of anhydrous potassium sulphate and copper sulphate and to distil off ammonia with caustic soda and arrest it in standard acid.

Bal [1925], while working on black cotton soils of the Central Provinces, found that a higher figure for nitrogen was obtained by 'Wet' digestion method than by the original 'Dry' digestion method. This was later con-

firmed by Sreenivasan [1932].

In our recent studies on the losses of nitrogen from soils it was considered necessary to standardize the method for the determination of organic nitrogen. For this purpose a trial experiment was conducted with two samples of soil, a sandy loam and a clay loam soil, and nitrogen determined by the original 'Dry' digestion method and the modified or the 'Wet' digestion method with 1:1 acid. The results obtained are given in Table I.

Table I

It will be seen from Table I that in sandy loam soil the figure for organic nitrogen was practically the same by both the methods. In clay loam soil, however, the 'Dry' digestion method gave higher results by about 7 mg. of nitrogen. The determinations were repeated with the same results.

The difference in results by the two methods could be attributed either to incomplete digestion of certain forms of nitrogen by the 'Wet' digestion method, which is unlikely and contrary to the existing evidence or due to the reduction of nitric nitrogen in the 'Dry digestion method. In order to test the latter point both the soil samples used in the previous experiment were separately treated with potassium nitrate solution to supply 50 mg. nitric nitrogen per 100 gm. of soil. These samples were air dried, and the nitrogen determined by both the methods. The results were as shown in Table II.

TABLE II Mg. N per 100 gm of soil

Method Nitrie Organic N Nitrie

Mg. N	per <b>1</b> 00 g <b>m.</b> of s	ioil	Soil	of digestion	N added	deter- mined	included
Soil	Dry digestion	Wet digestion	Sandy loam Sandy loam Sandy loam	Dry Dry Wet	Nil 50 Nil	28·70 38·50 29·40	9-80
Sandy loam	28.7 28.7	29.4 29.4	Sandy loam Clay loam Clay loam	Wet Dry Dry	50 Nil 50	28·70 78·4 92·4	Nil - 14:0
Clay loam	78.4 77.0	70.0 71.4	Clay loam Clay loam	Wet Wet	Nil 50	70-0 71-4	1.4

From Table II it will be seen that in both the soils a part of the nitric nitrogen added was reduced by the 'Dry' digestion method and that the reduction was greater in the clay loam soil than in the sandy one. On the above evidence it may be concluded that the higher results originally obtained for nitrogen in the untreated clay loam soil were due to the reduction of a part of the original nitric nitrogen (12 mg.) present in the soil.

The experiment was repeated with some other typical soils from different localities. The soils were treated with potassium nitrate at 50 mg. nitrogen per 100 gm. of the soil, and nitrogen estimated by both the methods. The results (Table III) confirmed the above finding, viz. that a part of nitric nitrogen is reduced by dry digestion. The reduction is comparatively greater in the Palampur soil which is rich in organic matter.

Table III

Mg. nitrogen per 100 gm. of soil

	Perc	entage	Meth dige	Nitric	
Soil	Clay	Organic matter	'Dry'	'Wet'	N in- cluded
Palampur	35	1.5	199.6	173.6	26.0
Dhundi estate	46	0.3	100.1	94.5	5.6
Montgomery	20	0.2	55.3	47.6	7.7
Sargodha	24	0.3	•57.4	46.2	11.2

In order to confirm the above results, another experiment was conducted as follows:

One hundred grams of the clay loam soil were shaken with 400 c.c. of distilled water for half an hour, transferred to a Buchner funnel and washed free of nitrates. The washed soil was air dried, sieved and organic nitrogen determined by both the methods. The results were as follows:

		•		Ma	NT.	10	10	:1
				Mig.	TA	per 10		· SO11
, Di	ry di	gestio	n.			69.	.3	
						69	3	
*-XX7	et 'di	igestio	n			<b>7</b> 0.	•	
	ct u	igeatio		•				
1.35 (1.5)		a bara carriera				70.	7	

From these results also it becomes clear that higher results for organic nitrogen obtained previously by the 'Dry' digestion method were due to the reduction of nitric nitrogen.

For turther verification of the results, the digestion of clay loam soil (original sample)

was conducted in a closed system. The evolved gases were slowly bubbled through soda and ferrous sulphate solutions. When the digestion was complete, purified air (free from ammonia and nitrous fumes) was aspirated for a few minutes. The contents of the absorption bottles were reduced with Devarda's alloy and nitrogen estimated. It was found that the gases from the 'Wet' digestion flask contained more nitrogen than those from the 'Dry' digestion flask (3.0 mg.) This showed that the oxides of nitrogen which are produced by the action of sulphuric acid on nitrates are partly fixed up in the course of the 'Dry' digestion.

## Conclusion

From the foregoing it becomes clear that a part of the nitrate nitrogen is reduced in the 'Dry' digestion method and therefore the results obtained for organic nitrogen are higher. The explanation seems to be that the nitrates are readily decomposed by the action of sulphuric acid whether dilute or strong with the evolution of nitrous fumes. In the 'Dry' digestion method, by the simultaneous action of the concentrated sulphuric acid on organic matter of the soil, numerous compounds are formed, side by side with the nitrous fumes and these exercise a reducing action on the nitrous fumes. While in the 'Wet' digestion method the nitrous fumes are boiled off before the acid is sufficiently concentrated to elaborate reducing substances.

The apparent conclusion from these studies is that the usual dry digestion method of the determination of organic nitrogen in soils gives higher results if the soil is rich in nitrates and that the 'Wet' digestion method is preferable.

In our trials, since 'Dry' digestion brought about the reduction of a part of nitric nitrogen even in case of a sandy soil which was very poor in organic matter, it is likely that a similar effect would be noticeable in other soils as well.

There is a seeming contradiction between our results and those obtained by Bal [1925] and Sreenivasan [1932], but it vanishes when we remember that they got lower results by 'Dry' digestion due to the incomplete digestion of their soils. The error due to this was very likely big enough to mask the effect of nitrate reduction as observed by us.

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# THE BIOLOGICAL DECOMPOSITION OF GREEN MANURES I. CARBON-NITROGEN TRANSFORMATIONS DURING DECOMPOSITION

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# Introduction

IN tropics, where animal manure is scarce, green manures form an essential feature in the system of crop husbandry employed in the growth of plantation crops. The maintenance of an adequate supply of organic matter in the soil carrying these crops depends largely on the frequent turning under of green manure crops.

Although green manuring offers a handy method in improving the organic matter status of soils yet it is highly complicated. Farming practice varies so much from place to place that success in extending the use of green manures depends first and foremost on an intimate knowledge of local conditions. The possibilities of green manuring, as of any other agricultural practice, will always vary from season to season. Such a tricky character of the practice of green manuring has been amply demonstrated by the failure of wheat and barley grown after mustard and tares in the famous Woburn experiments. A detailed account of these experiments is given by Crowther and Mann [1933].

Crowther and Mann [1933].

The subject of decomposition of green manures has attracted the attention of several workers on account of its complicated character. Studies have been made by Tenny and Waksman [1929], Crowther and Mirchandani [1931], Mirchandani [1931] and Daji [1934], using soil as the medium. The results of all these workers point to the same conclusions, viz. (a) that the age and nature of green manure plants have an influence on the amount and rapidity of liberation of nutrients, particularly nitrogen in an available form; and (b) unless a crop is ready to avail itself of such rapidly available nitrogen as ammonia or nitrate, the latter may soon get leached out during winter-rains and some

nitrogen may also be lost through volatilization.

As it is rather difficult to follow the changes in the soil because of the small quantities of nitrogen present for direct experiment, a parallel case was chosen where the biochemical decompositions are similar to those in the soil but the

quantities of nitrogen are relatively large. Such a method has been successfully adopted by Rege [1927], Norman [1929] and Shrikhande [1933, 1], for the study of several factors involved in the decomposition of materials like straws. These workers have followed the changes in nitrogen with respect to loss of dry matter with materials of a wide carbon-nitrogen ratio, but such direct study has never been reported so far for materials naturally rich in protein. Moreover, Howard [1935] has lately been advocating the composting of green manures instead of their direct incorporation on tea estates of Ceylon. It was therefore thought desirable to imitate the process of composting in the laboratory, under controlled conditions to see how far such a process compares with the age-long practice of green manuring. With this object in view the subject was followed up in considerable detail by studying the carbon-nitrogen changes in composts obtained and their respective hydrogen peroxide and water extracted residues as outlined below.

#### EXPERIMENTAL

A number of green manures with other vegetable materials used for composting on tea estates in Ceylon was rotted in presence of mixed natural flora for 35 days at room temperature of about 30°C., with the following changes in the nitrogen supply:

(a) Plant materials independently of any external source of nitrogen.

(b) Plant materials in presence of ammonium sulphate.

(c) Plant materials in presence of sodium nitrate.

(d) Comparitive tests on nitrification.

Original plant materials and their respective composts were extracted with water and hydrogen peroxide. Carbon and nitrogen determinations were conducted on all the samples.

#### TECHNIQUE AND METHODS

Twenty grams of oven-dry materials of known carbon-nitrogen ratios were fermented aerobically in bottles. Each bottle was given an inoculum from a compost so as to ensure a rapid and even growth of flora. Nitrogen in different forms

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was introduced at the rate of 1 gm. per 100 gm. of material and the moisture content was adjusted to about 70 per cent. Frequent stirring of the contents was resorted to in order to obtain uniform moisture distribution. This would avoid any chances of waterlogging. After the desired period each bottle was weighed with its contents and analysed as shown below:

1. Extraction with hydrogen peroxide—the method employed was described by Shrikhande [1933, 2]. The strength of peroxide used was

3 per cent.

2. Extraction with water—2 gm. of the vegetable material and its compost were extracted with the same volume of water and for the same length of time as with peroxide.

3. Ammonia and nitrate N were estimated by methods described by Shrikhande [1941].

4. Total N w s determined by the usual Kjeldahl method using sulphuric-salicylic acid mixture only where nitrate was present.

5. Organic C was determined by the Robinson, McLean and Rice Williams method [1929].

## RESULTS

Series I—Carbon-nitrogen ratios of various materials

Table I contains figures of carbon, nitrogen and C/N ratios of the plant materials. It will be noted from the data that the variation in carbons is less striking than in the nitrogen of the different plant materials. It appears as though the distribution of carbon is more even in this class of materials than nitrogen. Knowing therefore the approximate age of any such material it may be possible to roughly estimate its carbon content but not so with the nitrogen. On an average the value of carbon may be assessed at 40 per cent in a moderately aged material. The carbon-nitrogen ratios of all the materials except those of Maana grass, Grevillea, cane-reed and paddy straw approximate to a figure which is obtained by composting an average vegetable material. This seems to be the fundamental reason why such materials have been generally used as green manures on tea estates without precomposting them before incorporating them into the soil. Adhering therefore to the basic conceptions of composting, the principle of using such materials as green manure appeared to be quite sound. The fact that this conception has recently been called in question made further investigation of the decomposition of green manures desirable.

TABLE I

Carbon-nitrogen ratios of the unfermented materials

Material		Carbon	Nitrogen	C/N
Sun flower		36-91	3.37	10.96
Tephrosia		43.40	3.46	12.56
Refuse tea	••	42.73	3.97	10.75
Dadaps		40.62	2.54	15.97
Gliricidia	••	40.48	2.74	14.77
Maana grass		39.92	1.08	37.0
Tea leaf (prunings)		42-63	3.19	13.38
Weeds	••	34.99	2.01	17.43
Fern		41.01	1.79	22.88
Cane reed	••	36-92	0.96	38.50
Grevilleas		48•78	1.04	47.10
Paddy straw		34.59	0.78	44.12

Series II—Carbon-nitrogen ratios of residues obtained on water and peroxide treatment of plant materials

The object of such extractions was to see whether there existed any difference between the two extracting reagents and if so how one extraction differed from the other. Another reason for this series was to find out how much of the nitrogen in the plant material is easily available on the supposition that easily soluble nitrogen is readily available to the plant and micro-organisms because of its quick ammonification and subsequent nitrification Such an assumption appears reasonable since plants must obtain their nitrogen in soluble form. work of Heck and Whitting [1927], Heck [1929] and of Bedsole [1937] lends support to such a hypothesis. Bedsole [1937] analysed a number of weeds common to Florida for their water soluble and total nitrogen contents and also studied their nitrification in the soil. He found that water soluble nitrogen was the most important factor in promoting nitrification followed by total N. If the water soluble N

was 0.5 per cent or above, favourable nitrate accumulation occurred, even though the total N was less than 1.7 per cent. Plants above 1.7 per cent total N but less than 0.33 per cent water soluble N had a slow accumulation of nitrate in the soil.

Water extraction. In materials of a low carbon-nitrogen ratio, it will be seen from Table II that Tephrosia and Dadaps gave the lowest extracts. The foliage leaf gave a lower extract than refuse tea, which is a product of manufacture from younger leaf. All materials with a wide carbon-nitrogen ratio gave extracts lower than materials rich in protein.

Table II

Extraction of unfermented material with  $H_2O \not \in H_2O_2$  (Residues)

Material	$\mathbf{H_2}$	$H_2O_2$
Sun flower	77.5	65.5
Tephrosia	81.0	79.2
Refuse tea	70.2	72.0
Dadaps	81.2	71.8
Gliricidia	718	58.8
Maana grass	82-2	75.4
Tea leaf	78-2	45.9
Grevilleas	91.8	58.7
Weeds	74.2	73.6
Fern	74.8	59.0
Cane reed	83.1	76.5

Carbon-nitrogen ratios of residues are included in Table III. When the data in Tables I and III are compared an estimate can be made of the losses suffered by carbon and nitrogen

during water extraction. The loss of carbon varies between 3 and 11 per cent for all the materials. Taking into account the criteria established by Bedsole [1937] for easy nitrification of plant materials it will be observed that all the materials of a low carbon-nitrogen ratio except Gliricidia fall into this category. All of them have a total N content over 1.7 per cent with a water-soluble N content very much in excess of 0.33 per cent and hence should be easily nitrifiable. Materials with a naturally wide carbon-nitrogen ratio do not conform to such a condition and hence are not liable to quick nitrification. This observation taken in conjunction with the principles of composting is strong evidence in support of the practice of green manuring with materials of a low carbon-nitrogen ratio. Carbon-nitrogen ratios of residues are practically of the same order as those of fresh materials, suggesting thereby that both carbon and nitrogen have been removed in a similar definite proportion.

Hydrogen peroxide extraction. Referring back to column 2 in Table II it will be noticed that the residues obtained are lower than those after water extraction, with the exception of refuse tea. This anomalous behaviour of refuse tea can be accounted for by the fact that considerable changes occur during tea manufacture from fresh tea leaf and that the tannin present very readily forms insoluble oxidation products even from a water extract, much more so in a peroxide extract.

Looking once more to Table III we can see that the loss of carbon with peroxide falls between 6 and 19 per cent. Maana as with water gives the lowest loss and tea leaf suffers the

Table III C:N ratios of residues on extraction of original materials with  $H_2O$  and  $H_2O_2$ 

Material		C/N of the I	H <sub>2</sub> 0 extracte (Kesidues)	d material	C/N of the $H_2O_2$ extracted material (Residues)			
			C.	N,	C/N	О	N	C/N
Sun flower			31.46	2.80	11.26	25.81	1.54	16.76
Tephrosia			34.98	2.50	14.03	35.30	2.35	15.02
Refuse tea			31.34	2.87	10.91	32.63	2.86	11.39
Dadaps			35.19	1.67	21 03	30.56	1.19	25.76
Gliricidia			33.06	2.51	13.15	25.33	2.39	10.62
Maana grass			36.42	1.06	34.41	33.40	0-73	45.50
Tea leaf			36-29	2.39	15.21	23.02	1.12	20.59
Grevilleas			28.10	1.66	16.92	28.84	1.73	16.67
Weeds			31.59	1.59	19.85	24.85	1429	19.31
Fern			32.86	0.84	39 23	30-26	0.60	50.44
Cane reed			42.01	1.03	40.86	21.21	0.36	63.24

highest. The extraction of nitrogen with peroxide is much more than in the case of water. Peroxide thus appears to attack the nitrogenous complex more than other constituents except in Gliricidia and weeds. The exceptional behaviour of proteins in Gliricidia continues to be the same as with water although greater carbon has been knocked off by peroxide. In the case of weeds, extraction with water and peroxide produced identical results in respect of both carbon and nitrogen. This is suggestive of the difference between the proteins of Gliricidia and weeds from proteins of other tissues. Due to the higher extraction of protein with peroxide the carbon-nitrogen ratios of residues are higher than the parent materials, with the exception of weeds.

If the analogy of quick nitrification in relation to water-soluble N and total N is stretched a little further it may be suggested that the peroxide soluble N may form the upper limit for this nitrogen to become available by mineralization since it has been shown by Iyer et al. [1934] that oxidizing agents present in the soil assist in the liberation of plant food.

From the data presented in Tables II and III the difference between the strengths of water and peroxide as extracting reagents has been well established. Hydrogen peroxide has removed more of both carbon and nitrogen than water.

Series III—Nitrogen transformations with respect to losses of dry matter during decomposition

Results of analysis are given in Table IV. Fungus mycelium appeared on the 3rd or the 4th day and active growth was noticeable after a week with the exception of maana and fern with no supply of artificial nitrogen. This is obviously due to the low nitrogen content of the two materials. Every bottle when stirred smelt profusely of amonia after a fortnight's decomposition barring materials of a wide carbonnitrogen ratio and of tea leaf without any treatment although it has a narrow carbon-nitrogen ratio. This observation was confirmed by the actual amounts of ammonia recovered on distillation. But the absence of even a trace of ammonia from tea leaf is highly striking when compared to the behaviour of other protein-rich materials. Refuse tea which is a product of manufacture of tea flush produced some ammonification but this may be due to some modifications caused during manufacture. These observations have been confirmed by repetition. Sun-flower although so rich in protein also gave a low recovery of ammonia but this can be reconciled with the fact that it has only a small negative

nitrogen factor while fermenting alone and actually ends with a positive nitrogen factor [Richards and Norman, 1931] in presence of sulphate of ammonia. Tea leaf although it gave such a high negative nitrogen factor showed no signs of ammonification suggesting thereby that the loss of nitrogen is in the form of elementary nitrogen [Eden and Shrikhande, 1939]. This anomalous behaviour may be attributed to the presence of tannin in tea leaf. This aspect of the problem is examined in a greater detail in a separate communication. The very small positive nitrogen factor for maana may either be due to the absorbtion of ammonia from other bottles or it may be an experimental error. The losses of dry matter are normal and they seem to depend upon the nitrogen content of green manures.

Although there is no need of any external supply of soluble nitrogen for fermenting materials of a low carbon-nitrogen ratio, fermentations were conducted under such conditions by way of academic interest. It will be noted that sunflower, though it possesses more than enough protein for its decomposition has actually finished with a positive nitrogen factor in the presence of sulphate of ammonia. This observation finds support in the findings of Richards and Norman [1931], who also obtained a similar positive nitrogen factor with willow peelings with a high protein content. They have attributed this to the preferential utilization of ammonia over the plant protein. All the materials including tea leaf have produced ammonification. Such ammonification in tea leaf suggests that tea tannin has no effect on the ammonification of a material which is introduced externally but it does seem to interfere with the ammonification of its own protein suggesting some sort of association or linkage between the protein in the material. A fairly high recovery of ammonia on distillation in the case of Grevillea can be explained by taking into account the extremely refactory nature of the material as seen by the poor loss of dry matter of 7 per cent 'The complete recovery of total nitrogen with negligible losses of dry matter clearly denote that the microbilogical activity was insignificant and hence there was no material change both in the mineral and total All other materials of a wide carbonnitrogen nitrogen ratio finish with a positive nitrogen factor as anticipated. When the losses of dry matter with ammonium sulphate are compared with those with no treatment it will be noticed that they are lowered by an extra dose of nitrogen. This is in accord with the findings of previous workers.

There are two points which are worthy of

TABLE IV

Loss of dry matter and nitrogen transformation occurring during decomposition for 35 days

(Results expressed on 100 gm. original material)

. Mat	erial		Initial T.N.	Initial M.N.	Dry matter	Final T.N	NH <sub>3</sub> -N	N0a·N	Organic N	Nitroge factor
				l N	o Treatme	nt				
			3.37		58.4	1 3.38 (	0.14		3.25	· - 0·12
Sun flower	• •	••	3.46		59.0	3.52	0.56		2.95	- 0.50
Tephrosia		••	3.40		56.5	3.68	0.20		3.48	- 1.50
Refuse tea	• •	••	2.54		48.7	2.00	0.36		1.64	- 0.90
Dadaps	1.5		$\frac{2.54}{2.74}$		51.3	2.56	0.78		1.78	- 0.96
Gliricidia	. • •				67.6	1.09	•		1.09	+ 0.01
Maana grass			1.08		62.4	2.40			2.40	0.79
Tea leaf			3.19		49.0	1.81	••	(	1.81	- 0.20
Weeds		• •	2.01		49.0	1	and the second second			
Grevilleas		• • •	1.04		78.1	1.57	• •		1.57	- 0.22
Fern			1.79		78.1	1.91				- 0 22
Cane reed			0.96						••	• •
Paddy straw	••		0.78				•		•••	• •
				Ammor	iium sulph	ate				
			4.54	1.17	79.1	4.61	0.94	1	3.67	+ C·30
Sun flower		• •	4.57	1.11	69.0	4.28	1.56		2.72	- 0.73
Tephrosia				1.04	76.7	4.47	1.34		3.13	- 0.85
Refuse tea		****	5.01	1.11	80.0	3.34	1.01		2.33	- 0.21
Dadaps	• •	• • •	3.65	1.09	59.4	3 47	1 29		2.17	- 0.57
Gliricidia	• •	• •	3.83		66.3	0 19	0.91		1.28	+ 0.20
Maara grass			2.18	1.10	68.4	€·54	0.78		2.68	- 0.51
Tea leaf		••	4.24	1.05	45.9	2.39	0.51		188	= 0.18
Weeds	•	•••	3.09	· 1·08		2.12	0.79		1.33	+ 0.28
Grevilleas ·		7	2.14	1.10	92.9	2.61	0.92		1.17	- 0.10
Fern			2.87	1.08	75.6	1.66	0.48	*	1-17	+ 0.21
Cane reed		1	2.06	1.10	65.3	1.68	0.08		1.00	+ 0.22
Paddy straw		••1	1.87	1.09	75.1	1 7.08 1	0.09		1 -00	) T 0 22
				Sodi	um nitrate					
			4.49	1.12	66.8	3.19	0.42		2.77	<b>—</b> 0 60
Sun flower		•••	4.57	1.11	71.6	3.04	0.51	0.26	2.27	- 0.19
Tephrosia -		••	5.01	1.04	74.6	4.58	0.61	0.72	3 25	- 0.72
Refuse tea		•	3.65	1.11	73.3	3.04	0.33	1.27	1.44	<b>-</b> 0·11
Dádaps	••	. ••	3.83	1 09	61.3	3.07	0.39	0.53	2.13	- 0.61
Gliricidia			2.78	1.10	59.7	1.69		0.47	1.22	+ 014
Maana grass		•	4.24	1.05	70.0	3.46		0.48	2.98	- 0.21
Гea leaf	••	01		1.03	57·0	2.14	0.16	0.04	1.94	- 0.07
Weeds		•••	3.09	1.03	94.7	194		0.86	1.08	+ 0-04
Grevilleas.		• •	2.14	1.10	67.5	2.22		0.43	1.79	
Fern			2.87		62.6	1.57		0.62	0-95	- 0.01
Jane reed			2.06	1.10		1.80		0.60	1.19	+ 0.4
Paddy straw			1.87	1.09	84.6	7.90		3 00		* * * * ***

note in the sodium nitrate series. Firstly, the recovery of mineral nitrogen is not so high as with ammonium sulphate. Secondly the nitrogen factors have a greater negative value. This can be attributed to the losses of nitrogen due to denitrification. Here again, the losses of dry matter are affected by an excessive doze of nitrogen as in the case of ammonium sulphate. The extremely poor decomposition of Grevillea both with ammonium sulphate and sodium nitrate indicate the extremely resistant nature of this

plant tissue. It should be incorporated into the soil with a proportionate dose of nitrogen, or else it should form a good resistant material to be composted with green manures.

# Series IV—Carbon-nitrogen ratios of composts

These ratios are contained in Table V. The recovery of carbon with all the three types of composting is practically of the same order. It is however lowered in general by 10 to 15 per cent when compared with the carbon content

of fresh materials. Nitrogen in composts without any treatment follows a similar course as the parent materials with an exception in tea leaf. The carbon-nitrogen ratios of all the composts are lower than those of original materials.

Table V Carbon-nitrogen ratios of fermented materials (35 days)

Material	Carbon	Nitrogen	C/N
N	o treatment		
Sun flower	21·97 25·87	3·38 3·52	6·49 7·35
Tephrosia Refuse tea	29.12	3.68	7.91
Dadaps	20.94	2.00	10.46
Glirîcidia	21.64	2.56	8.47
Maana grass	28.72	1.09	26.32
Tea leaf (prunings)	29.22	2.40	12.20
Weeds	14.16	1.81	7.81
Fern	30-99	1.57	19.72
Cane reed	••	••	
Paddy straw			
Amm	onium sulpho	ite	
Sun flower	26.07	i 4·61 j	5.65
Tephrosia	27.01	4.28	6.30
Refuse tea	24.01	4.47	5.37
Dadaps	* 30.79	3.34	9.22
Gliricidia	23.34	3.47	6.74
Maana grass	27.75	2.19	12.68
Tea leaf (prunings)	29.60	3.45	8.57
Weeds	14.22	2.39	5.96
Fern	28.50 24.10	$\begin{array}{c c} 2.61 \\ 1.66 \end{array}$	10.92 $14.55$
Cane reed	18.30	1.68	10.87
Paddy straw		1 00	10 37
$-$ . So $\epsilon$	lium nitrate		
Sun flower	23.42	3.19	7.33
Tephrosia -	28.27	3.04	9.30
Refuse tea	28.43	4.58	6.21
Dadaps	24.40	3.04	8.03
Gliricidia Magne gross	23.14	3·07 1·69	7.54 15.88
Maana grass Tea leaf (prunings)	26.84 28.21	3.46	8 15
Weeds	15 13	2.14	7.06
Fern	22.85	2.22	10 29
Cane reed	18.77	1.57	11-98
Paddy straw Isi			

The carbon recovery of composts obtained with sulphate of ammonia and sodium nitrate is in general the same as with no treatment, but the nitrogens are apparently higher than compost without any treatment on account of the mineral nitrogen added. The carbon-nitrogen ratios are thus narrower than those of original materials. Comparing sulphate of ammonia with sodium

nitrate rots it can be seen that the carbons for both are similar but not so with nitrogen. Nitrogen in sodium nitrate rots are lower due to losses on denitrification and hence the carbonnitrogen ratios are wider here than in ammonium sulphate rots.

In actual practice, an attempt should never be made to compost such materials in presence of additional mineral nitrogen. The carbonnitrogen ratios of composts obtained without any treatment are in themselves highly significant. Their ratios have become narrower than 10:1. The average ratio obtained on composting a fairly resistant material is usually of the order of 12: 1. Attempting to ferment materials of narrow C/N ratios is to involve oneself in an undesirable waste both of carbon and nitrogen which are so essential for the growth of plants and organisms. In fact to compost such a material is to defeat the very object of composting. The principle of composting really consists in fermenting materials of wide carbonnitrogen ratios in the presence of a suitable source of available nitrogen, so as to bring down the ratio to that of soil humus (near 10:1) which is the optimum for easy nitrification.

There are, therefore, only two ways in which such materials can be successfully used. (1) by composting them with some resistant materials, (2) using them as green manures by digging them into the soil. Ammonia which is liable to be lost under ordinary composting will be profitably utilized either by the micro-organisms in elaborating microbial tissue under the first head or the ammonia as such may be absorbed by the soil complex thus preventing the whole of it being lost on volatilization as the growing crop may use it in the form of nitrate. The first principle was successfully adopted by Brown and Smith [1929] in composting straw in presence of clover and unsuccessfully by Bagot [1936] by composting green manure alone.

Series V—Carbon-nitrogen ratios of residues obtained after extraction with water and peroxide of composts

These determinations were conducted only on composts obtained without any treatment.

Water extraction. When these residues after water extraction in Table VI are compared with those obtained from original materials, it will be noted that the residues from composts are lower than those obtained from the parent material. Such a difference can be accounted for by the fact that certain constituents in the original material have been partly rendered soluble and partly destroyed by the micro-organisms during the process of decomposition.

TABLE VI Extraction of fermented material with  $H_2$ 0 &  $H_2$ 0<sub>2</sub> (Residues)

		and the same
Material	H <sub>2</sub> 0	H <sub>2</sub> 0 <sub>2</sub>
Sun flower Tephrosia Refuse tea Dadaps Gliricidia Maana grass Tea leaf (prunings) Weeds Fern	51·51 56 0 60·7 42·8 42·9 54·8 56 0 32·6	26 2 44-3 34-0 33-7 34-2 43-0 31-9 19-1

By studying Table VII along with Tables V and III it will be seen that both the factors are at play in giving lower residues from composts. Carbons in Tables V and VII are in general of

the same magnitude showing that very little carbon is water soluble whereas in certain cases over 30 per cent of nitrogen has been rendered water soluble in composts. That is why the carbon-nitrogen ratios of residues have gone up in their values. If both the carbons and nitrogens in Table VII are compared with those in Table III it will be found that they are lowered by about 30 and 20 per cent respectively. Both these constituents are apparently lost during decomposition, viewing the water solubility of nitrogen in a compost as a test of quick nitrification and easy availability, as discussed previously, we can conclude that 30 per cent of nitrogen in such composts should become easily available to the crop.

Peroxide extraction. Comparing again peroxide residues of composts in Table VI with those in Table II of fresh materials it can be observed that there is a considerable difference

C/N ratios of residues on extraction of fermented materials with  $H_2 O$  and  $H_2 O_2$ 

			(H <sup>2</sup> O)			(H <sub>2</sub> O <sub>2</sub> )	
Materia		0%	N%	C/N	C/N	N%	C%
Sun flower Tephrosia Refuse tea Dadaps Gliricidia Maana grass Tea leaf (prunings) Weeds Fern		20·74 25·04 26·50 20·15 18·72 24·63 28·03 11·94 30·71	2·29 1·90 2·87 1·10 0·97 1·20 2·03 1·20 1·57	9·04 13·20 9·22 18·28 19·22 20·56 13·84 9·91 19·54	9·73 18·61 14·88 17·23 14·83 19·07 13·39 6·30 21·69	0.74 1.56 2.30 0.83 0.50 0.63 1.25 0.30 0.99	13·18 11·91 6·48 20·71 29·76 30·43 10·71 21·44 21·80

between the two. That peroxide extracts much more than water can be cleary seen by comparing their respective residues in Table VI.

paring their respective residues in Table VI.

Arguing on similar lines as in Series II, we can say that peroxide extracted more of carbon and nitrogen from composts than water. About 25 to 30 per cent of carbon has been removed and nitrogen extracted in several cases has exceeded 60 per cent. Comparing the data in Tables III and VII it can be seen that approximately 40 per cent of carbon and nearly an equal amount of nitrogen have been apparently lost during decomposition of most materials of a narrow carbon-nitrogen ratio. 60 per cent which is shown to be peroxide soluble N may be taken as the upper limit for available N in composts since oxidizing agents present in the soil assist in the liberation of plant food [Iyer et al. 1934].

The figure quoted by various workers on the availability of nitrogen in composts and farmyard minure ranges between 33 and 68 per cent. These values were arrived at either by following the ammonification and nitrification and changes in soil with a minure or by the amount of nitrogen recovered in the growing crop. Tuxen [1884] for instance recovered only 33 per cent of N in farmyard manure as nitrate in five months. Iversen [1927] recovered 26-68 per cent over a period of eight years in his wheat crop and Popp [1908] noted that six weeks were required to nitrify 33 per cent N in manure.

All the above data also clearly demonstrate the greater action of peroxide than water over both composts and fresh vegetable tissues. These findings thus contradict the observations of Richardson [1931], who noted practically no

difference between the two extracting reagents.

Series VI—Comparison of the rate of nitrification of different materials

All the materials were ground to pass 64-mesh sieve. Nitrification tests were conducted at room tempetrature of about 25°C. The amount of N in each container was adjusted to 40 p.p.m. and each treatment was quadruplicated. 100 gm. of sand was taken in each container and the moisture content was maintained at 10 per cent. After seven weeks all the cultures were tested for mineral N by the Mclean and Robinson method [1924]. The results are summarized in Table VIII. It is interesting to note that the lowest limit of N for nitrification from this data is set at 1.79 per cent which is in accord with the well establishd concept. Lyon, Bizzell

and Wilson [1923] showed that nitrate is taken up from the soil during the decomposition of plant residues containing less than about 18 per cent N: this amount, however, seemed to keep the process self-supporting. Materials containing more N than this increased the NO<sub>3</sub> content of the soil. This observation is also in keeping with the results in Table IV on the decomposition of materials in bottles when materials below 1.79 per cent have not undergone any change without an external supply of available N. The percentages of total nitrification appears to be proportional to the initial total N content of the materials with an exception in refuse tea which contains about 18 per cent tannin, but there seems to be no strict relation hip between nitrification and water-soluble N as suggested by Bedsole [1937].

# TABLE VIII

# Mineralization of nitrogen in original materials

1	Material		NO <sub>3</sub> p.p.m.	Nitrification per cent of total nitrogen	Percentage of total N in the material	Percentage of H <sub>2</sub> 0 soluble nitrogen	Percentage of H <sub>2</sub> 0 <sub>2</sub> soluble nitrogen
Sun flower		••	8.29	20.68	3.37	0.57	1.83
Gliricidia			9.29	23.24	2.74	0 23	0.35
Tephrosia			17.20	43.00	3.46	0.96	1.11
Dadaps			2.80	6.99	2.54	0.87	1.35
Refuse tea		••	2.24	5.60	3.97	1.10	1.20
Weeds		•••	6.03	15.09	2.01	0.35	0.28
Fern		•••	4.70	11.74	1.79	0.20	0.50
Maana grass					1.08	0.02	0.35
Grévilleas		••/			1.04	0.01	0.67
Cane reed					0.96	0.15	0.36
Paddy straw					0.78		

## SUMMARY AND CONCLUSIONS

Decomposition of green manures and other suitable material for composting on tea estates in Ceylon has been studied with respect to their carbon-nitrogen changes, with the following observations:

(1) The composition of various materials examined shows that the carbon nitrogen ratio of all the green manures is in the neighbourhood of 12:1, a ratio obtained in a compost of an average vegetable material. Some uniformity in the distribution of carbon in these materials has been suggested

(2) Hydrogen peroxide produces a greater loss on extraction of fresh materials than water. Peroxide extracts more of carbon and nitrogen than water. The carbon-nitrogen ratios of residues on water extraction are of the same order as of the original materials, but not so with peroxide residues.

(3) All the green manures have finished with a negative nitrogen factor with ready ammonification of the plant protein, with the exception of tea leaf. The leaf with its normal decomposition and negative nitrogen factor, failed to produce any ammonia, suggesting that the loss is perhaps due to elementary nitrogen. The possibility of interference due to the presence of tannin in tea leaf has been sugge ted. Sun flower although rich in nitrogen has nevertheless immobilized some ammoniacal nitrogen and finished with a positive nitrogen factor.

(4) Carbon-nitrogen ratios of all the composts from green manures are narrower than those of parent materials. The futility of composting such green manures has been explained on the basis of nitrogen factors as tremendous losses of nitrogen occur during such a process. Two profitable ways of using such materials

have been indicated.

(5) Hydrogen peroxide produces a greater loss on extraction of composts than water. Both carbon and nitrogen are lost in greater amounts with peroxide. The carbon-nitrogen ratios of peroxide residues are narrower than the composts. About 30 to 60 per cent of nitrogen which is attacked by peroxide in composts may be taken as the amount which may become available to the plant by mineralization.

(6) Comparative tests on nitrification indicate a relationship between total nitrates formed and initial total nitrogen content of plant residues but no strict relation is traceable between nitrification and water-soluble nitrogen as suggested by Bedsole [1937]. 1.79 per cent N in fern sets the low st limit to nitrification in the series tested. This limit agrees with the well established concept that about 1.8 per cent N in a material keeps the process of nitrification self-supporting.

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# PROPERTIES OF SYNTHETIC MIXTURES

#### MIXTURES OF COLLOIDAL SOLUTIONS OF SILICIC ACID, ALUMINIUM II. HYDROXIDE AND FERRIC HYDROXIDE\*

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(With two text-figures)

THE electrochemical properties of mixtures of colloidal solutions of silicic acid and aluminium hydroxide have been discussed in Part I of this paper [Chatterjee and Sen, 1943, 1]. The present paper \*\* deals with the properties of synthetic mixtures of silicic acid, aluminium hydroxide and ferric hydroxide sols

The methods of preparation of colloidal silicic acid and aluminium hydroxide have been

Ferric hyreported in the previous part. droxide sols were prepared by adding. drop by drop, a saturated solution of ferric chloride to boiling water The hydrochloric acid generated in the process and the unhydrolysed ferric chloride, if any, were removed by dialysis. Final purification was effected by electrodialy-

Two mixtures were prepared by mixing the pure sols in different proportions. Mutual coagulation was noticed when the sols were mixed and the coagulated mass settled down after some time.

(a) Chemical composition

Percentages of silica, alumina and ferric

<sup>\*</sup> The work has been carried out under the Scheme of Research into the Properties of Colloid Soil Constituents' financed by the Imperial Council of Agricultural Research. Prof. J. N. Mukerjee is the Director of the Scheme.

<sup>\*\*</sup> A note on this subject has been published in the Proceedings of the Indian Science Congress Association,

oxide in the mixtures and their silica-sesquioxide ratios are shown in Table I.

#### TABLE T

Mass-chemical composition of over dried mixtures of silica, alumina and ferric oxide

System	Si0 <sub>2</sub> %	A1203%	Fe203%	Si0 /F203 (molar)
Mixture I	60-11	23.72	15 21	3.10
Mixture II	51.22	36.02	11.96	1.95

# (b) Effect of ageing on pH and conductivity

Variations in the pH and specific conductivity of mixtures I and II with time are shown in Tables II and III.

TABLE II

Variations in the pH of synthetic mixtures with time

	d gm.	1				
System				$p\mathbf{H}$	٤.	
System	Collo   content   per litr	17-3-41	19-3-41	20-3-41	25-3-41	30-3-41
Mixture	4.3	4.50	4.23	4.21	4.03	4.05
Mixture 11	4.3	<b>4</b> ·30	4:30	4.18	4.08	4•11

TABLE III

Variations in the specific conductivity of synthetic mixtures with time

	Specific cond. × 105 mho.								
System	17-3-41	19-3-41	20-3-41	25-3-41					
Mixture I	4.61	5.08	5-20	5.25					
Mixture II	4.45	4.81	5•00	5•02					

The data cited in Tables II and III show that ageing has a definite effect on the pH and conductivity as also observed with the binary mixtures of colloidal silica and aluminium hydroxide previously studied [Chatterjee and Sen, 1943, 2]. The variations of the pH and conductivity indicate a slow interation between the colloidal solutions. In the case of mixtures I and II the pH decreases and the specific conductivity increases with time (Tables II and III) but the

mixtures previously studied showed an increase in both the pH and specific conductivity. Ray chaudhuri and Mian [1943] consider that ageing favours a mineralization of the mixed gels.

#### (c) Interaction with alkalis

The potentiometric titration curves (Fig. 1) of the mixtures with NaOH differ from that of any of the ingredients in that the former show a definite inflexion point between pH 7.0 and 76 which is lacking in the titration curves of the pure systems [Chatterjee, 1939; Datta, 1939; Mukherjee et al., 1942]. Similar observations were made with the binary mixtures and also with some hydrogen clays previously studied in this laboratory [Chatterjee & Sen, 1943; Mukherjee et al., 1942]. Puri, et al. [1944] have recently observed a similarity of the titration curves of soil colloids with those of synthetic mixtures of ferroaluminium silicates. The inflexion is sharper the higher the Si02/R2O3 ratio of the mixture (Fig. 1). The form of the titration curve and the inflexion point suggest that some sort of interaction takes place on mixing the three soils.

pH and total acids calculated from titration curves are shown in Table IV which also includes similar data\* on a hydrogen clay L (isolated from a lateritic soil) having the same mass chemical composition as mixture II.

Table IV

pH and total acids of synthetic mixtures

System	pH	Si0 <sub>2</sub> /		id, milli- ents per gm.	pH at
		R 0 2 3	At inflexion point	At pH 7.0	inflexion
Mixture I Mixture II Hydrogen clay sof L	4·05 4·11 4·80	3·10 1·95 1·95	23·0 24·0 15·5	23·0 11·0 5·5	7·0 7·65 8·20

As usually found with hydrogen clays isolated from soils, the amount of the acid neutralized at  $pH\ 7\ 0$  increases with the  $SiO_2/R_2O_2$  ratio of the mixture. At the inflexion point, however, both the mixtures give almost the same total acidity.

Mixture No. II gives a greater total acid (calculated at the inflexion point, or at pH 7.0) than hydrogen clay L having a similar chemical composition. A higher pH at inflexion is found for the hydrogen clay, compared with this mixture.

(d) Buffer capacities

The potentiometric titration curves resemble that of a weak acid in true solution. It was,

<sup>\*</sup> Taken from a paper by Dr R. P. Mitra [1942]

therefore, thought desirable to study their buffer-capacity curves with a view to having fuller information on this point. Buffer capacities  $(\triangle B/\triangle pH)$  calculated from different points of

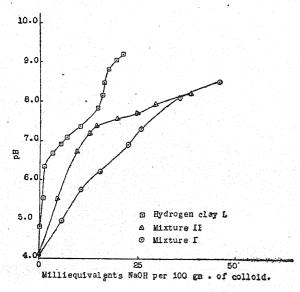


Fig. 1. Titration curves of the mixtures and hydrogen clay L with NaOH

the titration curves are plotted against the concentration of the added alkali in Fig. 2. On the addition of alkali, the buffer capacity gradually increases and after passing through a maximum and a minimum, it again increases. The maximum point in the buffer capacity curves does not correspond with the point of half neutralization

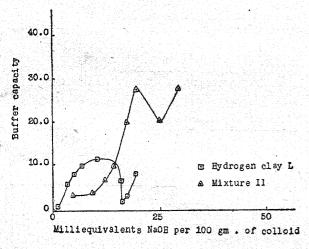


Fig. 2. Buffer capacity curves of mixture II and hydrogen clay L

but is shifted to a higher alkali concentration. The synthetic mixtures as also sol L differ from a dissolved weak acid in this respect. The similarity in the forms of the titration curves of these systems with that of a weak acid in true solution is, therefore, only superficial.

Mixture No. II shows a greater buffering than the hydrogen clay sol L (Fig. 2) though both have the same mass chemical composition. Similar observation was made by Bradfield [1923]. He found that a synthetic mixture of purified aluminium hydroxide, ferric hydroxide and silicic acid having the same composition as the colloidal material isolated from a heavy clay subsoil showed a stronger buffer capacity towards alkalis than the natural colloid.

(e) Liberation of Al ions from the mixtures by neutral salts

The supernatant liquids above mixtures I and II to which 0.09N BaCl<sub>2</sub> had been added were analysed for Al and Fe and the results are recorded in Table V. Similar data on hydrogen clay L are also given in that table.

# TABLE V

Analysis of salt extracts of synthetic mixtures and hydrogen clay

Sio R 2 2 2 3 2 3 2 3 3 3 3 3 3 3 3 3 3 3 3	piaced p 100 gm
Mixture II 1.95 3.2 Ni	
Mixture I 3-10 4-2 Ni	Ĺ
Hydrogen clay sol L 1.95 12.0 Ni	į.

Appreciable quantities of Al are liberated from all three systems though the hydrogen clay gives a much larger quantity of Al than either mixture. No Fe could be detected in the supernatant liquid.

# SUMMARY

The pH and conductivity of two mixtures having different proportions of colloidal silica, alumina and ferric hydroxide change on ageing indicating a slow interaction between the ingredients.

Potentiometric titration curves of the mixtures with NaOH show a definite inflexion between pH 7.0 and 7.6 which is not found in the titration curves of any of the ingredients.

The total acidity at pH 7.0 calculated from the titration curve increases with Si0<sub>2</sub>/R 0<sub>3</sub> ratio

of the mixture but at the inflexion point both the mixtures give almost the same total acid. The pH at inflexion is lower the higher the SiO<sub>2</sub>/R<sub>2</sub>O<sub>3</sub> ratio.

One of the mixtures shows a greater buffering towards alkalis than a hydrogen clay having the

same mass chemical composition.

Appreciable quantities of Al are liberated from the mixtures on the addition of BaCl, (0.09N). Fe could not be detected in the salt extract.

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# STUDIES IN INDIAN CEREAL SMUTS VII. FURTHER STUDIES IN VARIETAL RESISTANCE OF INDIAN AND OTHER WHEATS TO LOOSE SMUT

By B. P. Pal and B. B. Mundkur, Imperial Agricultural Research Institute, New Delhi (Received for publication on 12 December 1944)

I<sup>N</sup> a previous communication the authors [Mundkur and Pal, 1941] gave the results of testing nearly 100 varieties of wheat against a race of loose smut originally collected at Pusa. Subsequent studies indicated that there are probably two races of loose smut in this country The tests with the original race, which will for convenience be referred to as L<sub>1</sub>, were therefore continued, while at the same time the varieties were also tested for their reaction to the second race which will be referred as L2. The latter

695-702; 13: 54-58, 631-33

The first article of this series appeared in the Proc. Indian Acad. Sci. 9: 267-70 (1939), and the remaining ones in the Indian J. agric. Sci. 11: 675-86, 687-94;

was originally collected at Delhi in 1939. The complete results for the period of the investigations (1937-1944) are set forth in Table I. With the increase in the amount of material handledl it was not possible to test all the varieties in ale the years. Taken collectively, however, thresults constitute a statement of relative resist ance of important Indian and foreign varieties which should be of interest and value to wheat breeders in India, and possibly also to those working in other countries.

The methods of infecting the ears, etc. are identical to those employed before and described in the communication referred to above, and hence they are not again described here.

# TABLE I Reaction of wheat varieties to two races of loose smut

Variety	Reaction to race $L_1$ Percentage of smutted plants in						Rection to race L <sub>2</sub> Percentage of smutted plants in					
Taylord, Survivors	1937- 38	1938- 39	1939- 40	1940- 41	1941- 42 د	1942- 43	1943- 44	1939- 40	1940- 41	<b>1</b> 941- 42	1942- 43	1943- 44
Indian varieties Imperial Pusa 4 F , , , 12 C s, , , 52	23·0 58·4 30·8	92·4 94·4 91·8	67·0 62·7	34·8 12·6				28.0	36·6 78·3	0•0 3•1	38·7 64·1	29.1 50·4 17·4

Table I (contd.)

Var	iety		Reaction to race $L_1$ Percentage of smutted plants in						Reaction to race L <sub>2</sub> Percentage of smutted plants in				
		1937- 38	1938-	1939- 40	1940-	1941- 42	1942-	1943- 44	1939- 40	1940- 41	1941- 42	1942- 43	1943
Imperial Pusa	80-5	30.5	23.0	74.1	21.5				-	21.6	25.4	26.6	10.0
" "	101	80.8	91.0	90.6					88.3	86.6	41.9	63.8	16·0
" "	111 114 *	29.2	100.0	38.1	40.5					63.3	0.0	47.1	25.9
" "	120	9.7	0.0	0.0	0.0				0.5	6.8	0.0		
,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	121 /	0.4	0.0	4.5	0.0				1	0.8	0.4	0.0	0.0
,, ,,	122	20.6	0.0	0.3	0.0				1.1	4·3 2·7	0.0	0.0	2.3
" "	123	11.4	91.4	j						2-1	0.0		100
21 22	124	0.0	0.0	0.0	0.0				9.3	2.0	0.0		•
" "	$125 \\ 126$	14.2	64.5	14.9	34.3					- 50.0	0.6	51.4	23.5
" "	163-3	19.7	84.9	0.0	0.0			12 1					
" "	163-4		0.0	0.0	0.0	0.0				1.8	0.0	0.0	3.8
23 21	165 ,		0.0	17.4	0.6	0.0			5.3	2.6	0-0	55.8	3.1
" "	114-1-8	1		0.0	0.0	"			0.,	20	0-0	0.0	0.0
" "	120.7				0.0				0.0	0.0			00
"	120-8 7				0.0				0.0	0.0			
Punjab "	120-19 - 8 A	72-0	93-1	40.1	0·0 5 <b>5</b> ·1	m= 0			0.0	0.0			
	9 D	13.9 45.5	100.0	49.1	99.1	75.8	45.2	26.4		70.1	53.5		53.5
<b>,</b>	C 518	52.5	96.4	66.3	34.1		57.2	68-4		83.4	7.5		50.9
<b>~</b> "	C 591	75.3	100.0	76.7	56.0	81.5	46.9	50.7		09.1	1.9		50.5
Cawnpore	13	15-1	84.3	33.7	59.3					52.7	3.3		5.03
Foreign of Federation (New s		0.0	45.0								-		
	d-glumed)	0.0	0.0	0.0	0.0				61.5	63.7	0.0		
	hite-glumed)	0.0	0.0	0-0	0.0	0.0			0·0 12·9	0·0 2·9	6.0		2.9
(from T	arnab)	0.0	0.0	0.0	0.0	0.0			4.6	6.2	0.0	0.0	0.0
Chinese White Flora			0.0	1:1	0.0	0-0	0.0	0.0	1	0.0			
Sword			0.0	0.4	0.0	0.0				0.0			
Khapli (T. dieoccu	992 \		0.0	0.0	0.0	0.0				0.0			
Free Gallipolli			0.0	0.0	0.0	0.0				0.0			
Igachikugo			0.0	0.0	0.0	\'.•				0.0			
Eshimashinraki			0.0	0.0	0.0	0.0				00,			
Ford Dundee			0.0	0.3	0.0	0.0				1.6	0.0		
Florence			0.0	0.0	0.0	0.0				0.0			
C 5271-W1			0.0	$\frac{0.0}{18.7}$	0·0 15.1	0.0	0.0	0.0	-1	0.0			
Kubanka (T. duru)	n)	1 3-5-44		10.7	0.0	22·1 0·0	0.0	0.0					
Reliance					0.0			0.0	Albert (			0-0	0.0
Mediterranean					0.0				4				
Hope Kenya (E 144)		H 23.31			0.0			0.0				0.0	0.0
" (E 148)		Hereni		4374	5·5 1·6	1.4	5.0	8.6		i		14.9	28.9
" (E 220)					0.0	24.0	2.9	60·2 0·0				0.9	0.6
Thatcher					0.0			UU				09	υυ.
Ardito					6.6	14.6	0.0	12.7	ì			2-0	6.4
Mentana				191 194	17.8	13.2	26.1	2.8				9.0	9.1

The results show that, generally speaking, various which are susceptible to race  $L_1$ . of loose smut are also susceptible to race  $L_2$ . although the degree of susceptibility may differ. Several varieties which are immune from or highly resident to  $L_1$  are slightly susceptible to  $L_2$ ; with the doubtful exception of Chinese

White there seems to be no case, however, where a variety immune from or highly resistant to L<sub>2</sub>. is susceptible to race L<sub>1</sub>. While L<sub>1</sub> may be restricted to Eastern India and L<sub>2</sub> to Northern India, it is obvious that the wheat breeder must take both the races into account when breeding wheats for loose smut resistance and it is

fortunate that a number of wheats are immune from or highly resistant to, both the strains of loose smut. These include the commercially important Imperial Pusa 114, 120, 165 and Khapli, the following new or little-tried strains bred at the Imperial Agricultural Research Institure: IP 121, IP 122, IP 124, IP 163-3, IP 163-4, IP 114-1-8, IP 120-7, IP 120-8, and IP 120-19; also a large number of imported varieties including Federation (three strains out of four in our collection), the rust resistant Kenya wheats (E 114, E 148 and E 220) and Reliance.

Besides the varieties enumerated in Table I, 62 new strains of hybrid origin, bred in the Botanical Section of this Institute, have been tested. Of these, 22 proved immune from or very highly resistant to both the smut races, 12 proved very highly resistant to L<sub>1</sub> alone (six of these how ver were not tested at all against L2), one proved very highly resistant to L2 (this was not tested against L1) while the remainder showed varying degrees of susceptibility ranging from about 5 per cent to 75 per cent.

Thus there are a large number of loose smut resistant strains already available in India and these constitute ample basis for building up new wheats combining loose-smut resistance with other desirable characters.

# SUMMARY

1. The reaction of 26 Indian varieties and 25 foreign varieties of wheat to two races of loosesmut commonly occuring in India is given in detail. The results of tests with 62 new strains of hybrid origin are also briefy summarized.

2. Nearly 50 per cent of the varieties tested, including some strains which are not distributed to farmers, proved to be resistant to both the strains of loose smut. The remainder showed varying degrees of susceptibility to one or both the smut strains.

3. It is pointed out that there is ample material for breeding new wheat varieties combining resistance to loose-smut with other desirable characters.

#### ACKNOWLEDGEMENTS

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Mundkur, B. B. and Pal, B. P. (1941) Studies in Indian Cereal Smuts, II. Varietal resistance of Indian and other wheats to loose smut Indian J. agric. Sci. 11.

# STUDIES IN INDIAN CEREAL SMUTS NOMENCLATURE OF INDIAN SMUT FUNGI AND PROBABLE MODES OF THEIR TRANSMISSION

By B. B. MUNDKUR, Imperial Agricultural Research Institute, New Delhi (Received for publication on 21 December 1944)

IN a recent publication Stevenson and Johnson [1944] have reviewed the position regarding the names that should be applied to cereal smut fungi in order to strictly adhere to the International Rules of Botanical Nomenclature.

A majority of the Mycologists will be in complete accord with the conclusions of Stevenson and Johnson [1944]. Their list of names deals with only those smuts that occur in the U.S.A. A list which includes the smuts affecting the Indian crop plants has therefore been compiled and presented below.

The list also includes the available information regarding the modes of transmission of the smut diseases, wherever they are known. In the study of plant diseases, a knowledge of their mode of transmission is very important, for without such knowledge, it is not possible to devise proper control measures. Smuts may be externally seed-borne or internally seed-borne, soil-borne or air-borne. Methods developed for controlling externally seed-borne smuts are worthless for controlling internally seed-borne smuts. For smuts that are air-borne seed treatments are of no value.

Unfortunately we do not yet know the mode of transmission of all the smuts affecting our crop plants. Recently the author [1943] has shown that the Karnal bunt is air-borne. Probably the bunt of rice is also air-borne. It is hoped that the Mycologists in whose areas such smuts occur will be stimulated to do further research so as to fill the lacunae in our knowledge of the transmission of such smuts.

Previous contributions in this series appeared in Proc. Indian Acad. Sci. 11, 267-70 (1939); Indian J. agric. Sci. 11, 675-702 (1941); 13, 51-58, 631-633 (1943)

		Scientific nar	no according to	
Crop	Common name of smut	International Rules of Botanical Nomenclature	Previous practice	Mode of transmission
Wheat (Triticum spp.)	Loose smut	Ustilago tritica (Pers.) Rostr.	Ust. tritici (Pers.) Jens.	Internally seed-borne, (floral infection)
	Flag smut	Urocystis tritici Koern.	No change	Externally seed-borne
	Karnal bunt	Neovossia indica (Mitra) Mundkur	Til. indica Mitra	Air-borne, floral_in fection
	Rough spored bunt	Tilletia caries (DC. Tul.	Til. Tritici (Bjerk.) Wint.	Externally seed-borne
	Smooth spored bunt	Tilletia foetida (Wallr.) Liro	Til. foetans (B. and C.) Trel. or Til. levis Kuehn	Externally seed-borne
Rice (Oryza sativa)	Leaf smut	Entyloma oryzae Syd.	No change	Not known
	Bunt	Neovossia horrida (Tak.) Padw. and Azmt.	Til. horrida Tak.	Probably air-borne (floral infection)
Jowar (Sorghum vulgare)	Grain or covered smut	Sphacelotheca sor- ght (Link) Clint.	No change	Externally seed-borne
	Loose smut	Sphacelotheca cru- enta (Kuehn) Potter	No change	Externally seed-borne
	Long smut	Tolyposporium eh- renbergii (Kuehn) Pat.	Toly. filiferum Busse	Probably air-borne (floral infection)
	Head smut	Sphacelotheca re- iliana (Kuehn) Clint.	Sorosporium reili- anum.	Probably soil-borne
Oats (Avena spp.)	Loose smut	Ustilago avenae (Pers.) Rostr.	Ust. avenae (Pers.) Jens	Externally seed-borne
	Covered smut	Ustilago Kolleri Wille	Ust. levis (Keller. and Sw.) Magn.	Externally seed-borne
Barley (Hordeum spp.)	Loose smut	Ustilago nuda (Jens.) Rostr.	Ust. nuda (Jens.) Kell. and Sw.	Internally seed-borne (floral infection)
	Covered smut	Ustilago hordei (Pers.) Lagerh.	Ust. hordei (Pers.) Kell. and sw.	Externally seed-borne
Iaizo (Zea mays)	Smut	Ustilago mays-zeae (DC.) Corda	Ust. zeae (Beckm.) Unger.	Air-borne
	Head smut	Sphacelotheca re- iliana (Kuehn) Clint.	Sorosporium reili- anum (Kuehn) McAlp.	Not known

보통하다 가는 경기 (1985) 통사람들의 분통 경기 (1985) 보통		Scientific nam	ne according to	
Crop	Common name of smut	International Rules of Botamical Nomenclature	Previous practice	Mode of transmission
Bajra (Pennisetum typhoides)	Smut	Tolyposporium pe- nicillariae Bref.	No change	Air-borne
	African smut	Tolyposporium se- negalense Speg.	No change	Unknown
	Bunt	Tilletia ajrekari Mundkur	No change	Probably air-horne
Kagni or rale (Setaria italica)	Grain smut	Ustilago crameri Koern.	No change	Externally seed-borne
Chena (Panicum mili-aceum)	Head smut	Sphacelotheca destruens (Sche.) Stev. and John.	Ustilago panicimi- liacei (Pers.) Wint.	Externally seed-borne
Sawan (Echinochloa frumentacea)	Rough spored grain smut	Ustilago panici- frumentacei Bref.	No change	Not known
	Smooth spored grain smut	Ustilago paradoxa Syd. and Butler	No change	Externally seed-borne
	Inflorescence smut	Ustilago crus-galli Tracy and Earle.		Not known
Ragi or Nachni (Eleu- sine coracana)	Grain smut	Melanopsichium el- eusinis (Kulk.) Mundkur and Thirumalachar	Ust. eleusinis Kul- karni	Probably air-borne
Kodra (Paspalum scro- biculatum)	Head smut	Sorosporium pas- pali McAlp	No change	Externally seed-borne
Kasi (Coix lacryma- jobi)	Grain smut	Ustilago coicis Bref.	No change	Not known
	Smooth spored grain smut	Ustilago lachryma- jobi Mundkur		Not known
Sugarcane (Saccharum spp.)	Stem smut	Ustilago scitaminea   Syd.	Ust. sacchari Ra- benh.	Through infected setts and nodes
	Covered smut	Sphacelotheca sac- chari (Rabenh.) Cif.	Ust. sacchari Ra- beuh.	Not known
Mustard ( <i>Brassica</i> spp.)	Root-gall smut	Urocystis brassicae Mundkur	Uro. coralloides Rostr.	Soil-borne

# SUMMARY

A list giving the common names of smuts, their scientific names in conformity with the International Rules of Botanical Nomenclature and names according to previous practice, together with the probable modes of their transmission, has been compiled so as to standardize the names.

dize the names.

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